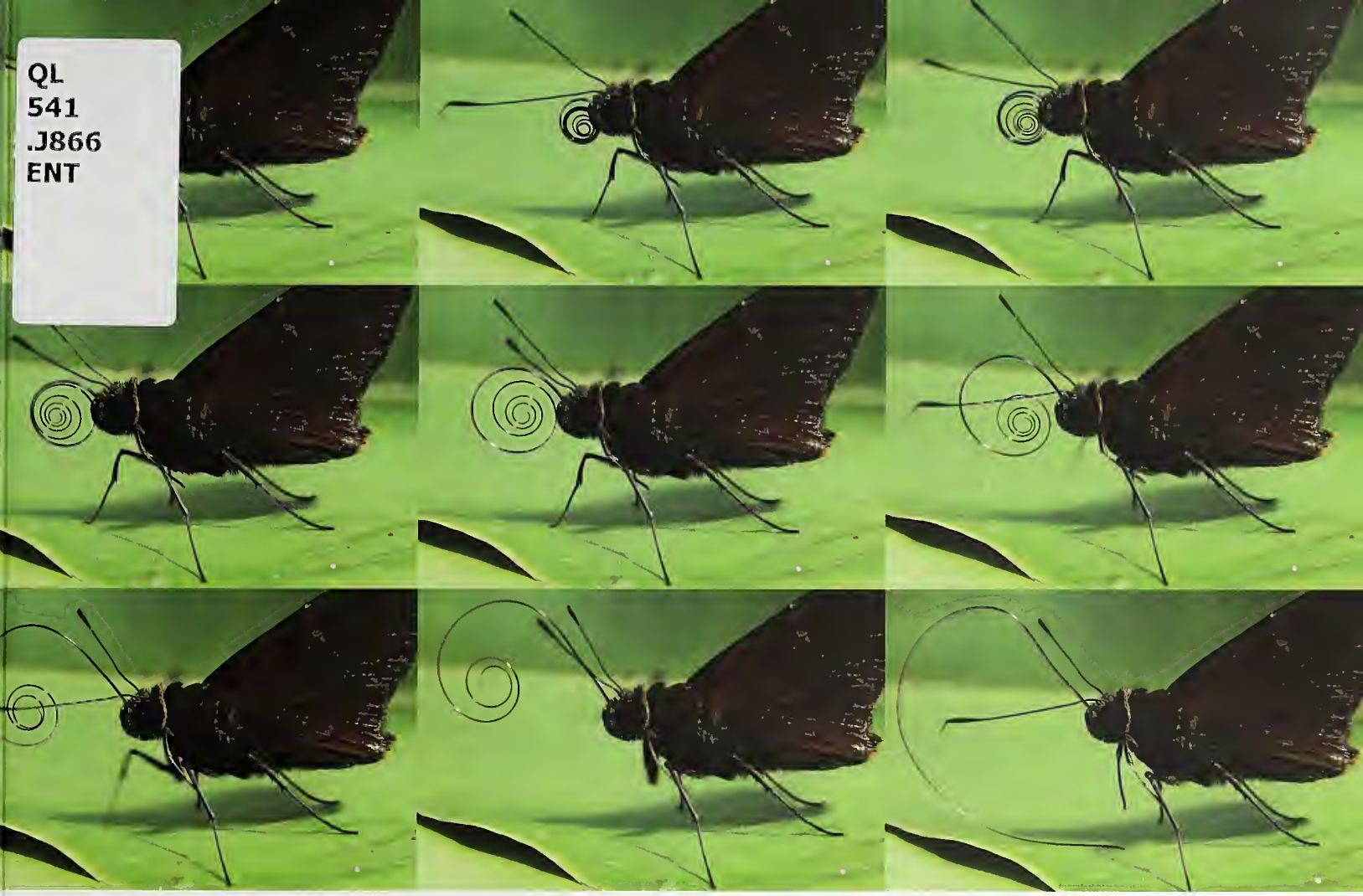

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Survey of the butterflies of the Sutter Buttes, California

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Abstract. The Sutter Buttes are a small, isolated mountain group in the center of northern California's Central Valley. Their location, nearly equidistant between the Coast Range and Sierra Nevada, make the Buttes biogeographically unique. Due to a history of private ownership public and scientific access to these mountains has been limited and much remains to be known about the natural history and ecology of the area. A previous survey of Sutter Buttes butterflies recorded surprisingly few species, and was suspected to represent an incomplete record of the butterfly diversity of the area. In order to assess the accuracy of this survey and explore the biogeographic relationships of the butterflies of north-central California, we performed surveys of the butterfly fauna of the Sutter Buttes. Over two years we performed bi-weekly transects and recorded species presence, abundance, and phenology as well as information about common butterfly host plants found there. Utilizing comparisons of transect data from the Coast and Sierra Nevada Ranges we found that the Sutter Buttes butterfly fauna more closely resembles the Central Valley floor fauna than that of either nearby mountain range. Our results also indicate that the Sutter Buttes harbor a significantly depauperate butterfly fauna: several butterfly species that are common at sites in the Central Valley, Coast Range, or the Sierra foothills are not present in the Sutter Buttes. We discuss possible reasons for these absences, including fire regime, host plant abundance, and nectar availability, and present

Key words: faunal survey, Sutter Buttes, island biogeography, California Central Valley

Introduction

The Sutter Buttes are considered to be the world's smallest mountain range. Located at 39.22 N 121.8 W, they rise from an elevation of 60 meters above sea level in California's Central Valley floor to 645 meters at their highest point, with several peaks over 480 meters (Hausback *et al.*, 2011). The Buttes were formed during a period of Pleistocene volcanism approximately 1.4-1.6 million years ago (Williams & Curtis, 1976). They are nearly circular, and are located almost equidistant from the Coast Range to the west and Sierra Nevada mountains to the east (Fig. 1). This combination of young age and insular location makes the Sutter Buttes interesting biogeographically, though for historical reasons much remains to be known about the ecology of the area.

The Coast Range and Sierra Nevada Range are good candidate source communities for the Sutter Buttes butterfly fauna. They share similar geology, climate, and floral and faunal communities with localities at similar elevations in the nearby ranges. The Central Valley, which lies between the two mountain ranges and surrounds the Sutter Buttes, is very different from either mountain range in terms of geology, climate, and butterfly community composition. For this reason it could be predicted that the Sutter Buttes butterfly fauna should differ from that of the Central Valley and be more similar to one or both nearby mountain ranges. In addition, since the Sutter Buttes are younger than the nearby ranges, colonization is more likely to be in the direction of immigrants to the Sutter Buttes rather than emigrants from the Sutter Buttes to either

* Both authors contributed equally to this manuscript

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nearby range (Schoenherr, 1992). A major aim of this study was to look for evidence of source communities from either the Coast Range, Sierra Nevada Range, or Central Valley floor.

The Sutter Buttes experience a Mediterranean climate characterized by long, hot summers and short, wet winters. The interior of the range receives an average annual precipitation of 51 cm, typically between December-February, compared to the surrounding valley floor, which receives 38 cm. The major ecosystem types of the Sutter Buttes are grasslands, chaparral, and oak woodland, with scattered wetland areas (Schoenherr, 1992). Prior to European colonization the surrounding Sacramento Valley was composed largely of tule marsh and underwent frequent flooding from the Sacramento River, the Feather River, several creeks from the Coast Range, and creeks from the Sierra Nevada. Most of the waterways have since been dammed, the valley drained, and the land developed for agriculture. See Anderson (1983, 2004) for a thorough review of the natural history and ecology of the Sutter Buttes.

While there are not believed to have been permanent human settlements in the Sutter Buttes prior to European colonization, several indigenous groups are known to have utilized the range for foraging, hunting, and cultural and religious reasons. The lands of three tribes, the Valley Maidu or Koncow to the north; the Valley Nisenan or Southern Maidu to the southeast; and Valley Patwin or the Wintun to the west and southwest, overlap at the Sutter Buttes. The largest impact of these indigenous peoples was likely their use of periodic fires for the purpose of native game and food plant management (Kroeber, 1925; Anderson, 2004). As with much of California, the region underwent early settlement by European trappers in the 1820's-1830's, followed by miners in the latter half of the century. The Sutter Buttes themselves were settled by Europeans in the late1800's and early 1900's for the purpose of grazing sheep and cattle, a practice that continues there today. The majority of the Sutter Buttes have remained privately owned since their European settlement, with public and scientific access limited or non-existent during the past half-century (Anderson, 1983). In recent years some private landowners have formed a partnership through the Middle Mountain Foundation to provide programs to promote public education and increase scientific access within the Buttes.

The purpose of this paper is to contribute to the current state of knowledge of the natural history and ecology of the Sutter Buttes by conducting regular surveys of the butterfly fauna present there. This survey aimed to record species presence, abundance, and phenology as well as host plant availability. In doing so we hoped to improve on a previous butterfly survey of the Sutter Buttes conducted over 30 years ago (Peoples, 1978, unpub. ms). Because Peoples found only 44 butterfly species (Table 1), a surprisingly small number relative to what might be expected based on the location of the Sutter Buttes, Peoples' survey was suspected to represent an incomplete documentation of the total butterfly diversity of the area. Additional aims of our study were therefore to assess the previous survey's accuracy, explore possible reasons why the Sutter Buttes might support such a depauperate butterfly fauna, and investigate whether observed regional declines are also detected in this isolated location.

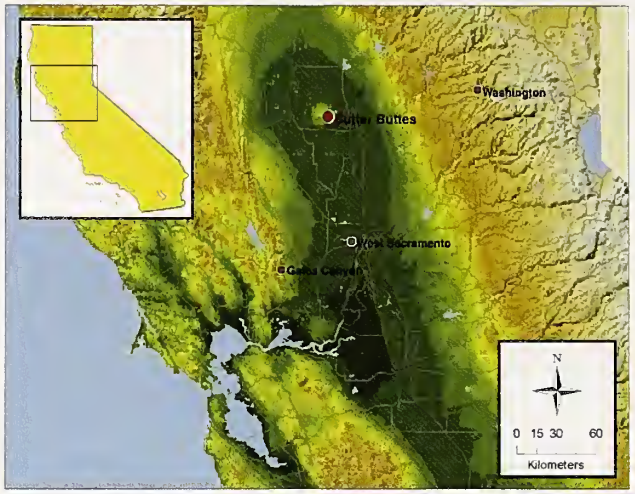

Figure 1. Location of comparison sites relative to Sutter Buttes, adapted from www.butterfly.ucdavis.edu

Methods

Study Site

This survey was conducted on the Dean Ranch property of the Sutter Buttes with the landowner's permission. This property has been family owned for over 100 years and is used primarily for grazing cattle and cultivating hay. The landowners have been instrumental in promoting educational and scientific access to the area, and much of the natural history information currently available about the Sutter Buttes has come from studies conducted on this site. Though there are structures and primitive roads on the property, much of Dean Ranch remains undeveloped and includes a variety of native ecosystem types. The site includes oak scrubland, a riparian corridor, grazed pasture, cultivated fields, and chaparral, and the terrain includes several hills, a large ridge, and is bordered to the north by the North Butte (Fig. 2).

Table 1. Species recorded at the Sutter Buttes and/or at the three comparison sites. Species recorded in the Sutter Buttes by Peoples, 1978 (SB1). Species recorded in the current study at the Sutter Buttes (SB2); Gates Canyon (GC); Washington (W); and West Sacramento (WS). '*' denotes species for which voucher specimens were collected in the current study (SB2); '#' denotes species for which the larval host plant is absent from the Sutter Buttes. Species in the family Hesperiidae were not included in comparison analysis. The total number of species counted during the current Sutter Buttes survey is included in the final column (Counts (SB2)). Counts are not available for Peoples' 1978 survey (SB1).

Family	Subfamily	Species	Site Presence	Counts (SB2)
Hesperiidae				
	Hesperiinae	*Atalopedes campestris* Boisduval 1852	SB1, SB2, W, WS, GC	NA
	Hesperiinae	*Hylephila phyleus* Drury 1773	SB1, SB2, WS, GC	NA
	Hesperiinae	*Lerodea eufala* Edwards 1869	SB1, SB2, W, WS, GC	NA
	Hesperiinae	*Ochlodes agricola* Boisduval 1852	SB1, SB2, W, GC	NA
	Hesperiinae	*Ochlodes sylvanoides* Boisduval 1852	SB1, SB2, W, GC	NA
	Hesperiinae	*Poanes melane* Edwards 1869	SB1, SB2, WS, GC	NA
	Pyrginae	*Erynnis propertius* Scudder & Burgess 1870 *	SB1, SB2, W, GC	NA
	Pyrginae	*Erynnis tristis* Boisduval 1852	SB1, SB2, W, WS, GC	NA
	Pyrginae	*Heliopetes ericetorum* Boisduval 1852 *	SB1, SB2, W, WS, GC	NA
	Pyrginae	*Pholisora catullus* Fabricius 1793	SB1, SB2, W, WS, GC	NA
	Pyrginae	*Pyrgus communis* Grote 1872	SB1, SB2, W, WS, GC	NA
Lycaenidae				
	Lycaeninae	*Lycaena arota* Boisduval 1852	GC, W	
	Lycaeninae	*Lycaena gorgon* Boisduval 1852	GC, W	
	Lycaeninae	*Lycaena helloides* Boisduval 1852	SB1, GC, W, WS	
	Lycaeninae	*Lycaena xanthoides* Boisduval 1853	SB1, GC	
	Polyommatinae	*Brephidium exilis* Boisduval 1852	WS, GC	
	Polyommatinae	*Celastrina ladon* Cramer 1780	W, GC	
	Polyommatinae	*Cupido comyntas* Godart 1824 *	SB1, SB2, WS, GC	7
	Polyommatinae	*Euphilotes enoptes* Boisduval 1852	W	
	Polyommatinae	*Everes amyntula* Boisduval 1852	W	
	Polyommatinae	*Glaucopsyche lygdamus* Doubleday 1841	GC, W	
	Polyommatinae	*Glaucopsyche piasus* Boisduval 1852	W	
	Polyommatinae	*Leptotes marina* Reakirt 1868	WS	
	Polyommatinae	*Philotes sonorensis* Felder & Felder 1865#	W	
	Polyommatinae	*Plebejus acmon* Westwood 1851 *	SB1, SB2, W, WS, GC	32
	Polyommatinae	*Plebejus icarioides* Boisduval 1852	W, GC	
	Theclinae	*Atlides halesus* Cramer 1777	SB1, SB2, W, WS, GC	2
	Theclinae	*Callophrys augustinus* Westwood 1852	SB1, SB2, W, GC	2
	Theclinae	*Callophrys dumetorum* Boisduval 1852	SB1, W, GC	
	Theclinae	*Callophrys gryneus* Hübner, 1819#	W	
	Theclinae	*Habrodais grunus* Boisduval 1852#	GC, W	
	Theclinae	*Incisalia eryphon* Boisduval 1852#	W	
	Theclinae	*Incisalia mossii* Edwards 1881#	W	
	Theclinae	*Satyrium auretorum* Boisduval 1852	GC	
	Theclinae	*Satyrium californica* Edwards 1862	SB1, W, WS, GC	
	Theclinae	*Satyrium saepium* Boisduval 1852	W, GC	
	Theclinae	*Satyrium sylvinus* Boisduval 1852	W, WS, GC	

Table 1. Continuation.

Family	Subfamily	Species	Site Presence	Counts (SB2)
	Theclinae	*Satyrium tetra* Edwards 1870	GC	
	Theclinae	*Strymon melinus* Hübner 1818	SB1, SB2, W, WS, GC	22
Nymphalidae				
	Danainae	*Danaus plexippus* Linnaeus 1758 *	SB1, SB2, W, WS, GC	15
	Limenitidinae	*Adelpha bredowii* Geyer 1837	SB1, SB2, W, GC	1
	Limenitidinae	*Limenitis lorquini* Boisduval 1852	SB1, SB2, W, WS, GC	5
	Heliconiinae	*Agraulis vanillae* Linnaeus 1758#	GC	
	Heliconiinae	*Speyeria callippe* Boisduval 1852	W	
	Heliconiinae	*Speyeria hydaspe* Boisduval 1869	W	
	Heliconiinae	*Speyeria zerene* Boisduval 1852	W	
	Nymphalinae	*Aglais milberti* Godart 1819	SB1, SB2,	1
	Nymphalinae	*Chlosyne palla* Boisduval 1852	W, GC	
	Nymphalinae	*Chlosyne leanira* Felder & Felder 1860	W, GC	
	Nymphalinae	*Euphydryas chalcedona* Doubleday 1847	W, GC	
	Nymphalinae	*Euphydryas editha* Boisduval 1852#	W	
	Nymphalinae	*Junonia coenia* Hübner 1822 *	SB1, SB2, W, WS, GC	51
	Nymphalinae	*Nymphalis antiopa* Linnaeus 1758	SB1, SB2, W, WS, GC	1
	Nymphalinae	*Nymphalis californica* Boisduval 1852	SB1, SB2, W, GC	4
	Nymphalinae	*Phyciodes mylitta* Edwards 1861 *	SB1, SB2, W, WS, GC	23
	Nymphalinae	*Phyciodes pulchella* Boisduval 1852	SB1, W	
	Nymphalinae	*Polygonia satyrus* Edwards 1869	SB1, GC	
	Nymphalinae	*Polygonia zephyrus* Edwards 1870	GC	
	Nymphalinae	*Vanessa annabella* Field 1971	SB1, W, WS, GC	
	Nymphalinae	*Vanessa atalanta* Linnaeus 1758	SB1, SB2, WS, GC	2
	Nymphalinae	*Vanessa cardui* Linnaeus 1758	SB1, SB2, W, WS, GC	72
	Nymphalinae	*Vanessa virginiensis* Drury 1773 *	SB1, SB2, W, WS, GC	1
	Satyrinae	*Cercyonis pegala* Fabricius 1775	GC	
	Satyrinae	*Cercyonis sthenele* Boisduval, 1852	W	
	Satyrinae	*Coenonympha tullia* Müller 1764	W, WS, GC	
Papilionidae				
	Papilioninae	*Battus philenor* Linnaeus 1771 *	SB1, SB2, W, WS, GC	564
	Papilioninae	*Papilio eurymedon* Lucas 1852	SB1, W, GC	
	Papilioninae	*Papilio multicaudata* Kirby 1884 *	SB1, SB2, GC	2
	Papilioninae	*Papilio rutulus* Lucas 1852 *	SB1, SB2, W, WS, GC	2
	Papilioninae	*Papilio zelicaon* Lucas 1852 *	SB1, SB2, W, WS, GC	1
	Parnassiinae	*Parnassius clodius* Ménétriés 1857#	W	
Pieridae				
	Coliadinae	*Colias eurytheme* Boisduval 1852 *	SB1, SB2, W, WS, GC	124
	Coliadinae	*Zerene eurydice* Boisduval 1855 *	SB1, SB2, W, GC	1
	Pierinae	*Anthocharis lanceolata* Lucas 1852	W	
	Pierinae	*Anthocharis sara* Lucas 1852 *	SB1, SB2, W, GC	8
	Pierinae	*Euchloe ausonides* Lucas 1852	SB1, GC	
	Pierinae	*Euchloe hyantis* Edwards 1871#	W	

Table 1. Continuation.

Family	Subfamily	Species	Site Presence	Counts (SB2)
	Pierinae	*Neophasia menapia* Felder & Felder 1859	W	
	Pierinae	*Pieris oleracea* Harris 1829	W, GC	
	Pierinae	*Pieris rapae* Linnaeus 1758 *	SB1, SB2, W, WS, GC	28
	Pierinae	*Pontia protodice* Boisduval & Leconte 1830	WS, GC	
	Pierinae	*Pontia sisymbrii* Boisduval 1852	W, GC	
Riodinidae				
	Riodininae	*Apodemia mormo* Felder & Felder 1859	W	

Transect

We conducted biweekly Pollard walks (Pollard, 1977) from March 2008 to March 2010, between 10:00-16:00 on days when weather was suitable to permit butterfly activity (typically 18-37° C, partial to full sun, low wind). A transect of 5-8 kilometers was surveyed which included several important habitat types, including riparian areas, hilltops, south-facing slopes, rocky outcroppings, and weedy fields (Fig. 2).

Butterflies were identified in flight, and some specimens were collected as vouchers. A total of 47 vouchers were taken, which are housed at the UC Davis Bohart Museum of Entomology. Many of the hesperiid species observed at the Sutter Buttes were located behind locked pasture gates and could not be captured or identified by eye; therefore the species list for Hesperiidae is almost certainly incomplete. We included them in the faunal survey only when they could be collected for identification, and we excluded members of the Hesperiidae from our analyses. To assess the likelihood that our survey recorded the majority of the butterfly species present in the Sutter Buttes, a randomized species accumulation curve (Gotelli & Colwell, 2001) was constructed using the 'Vegan' package in R (Oksanen *et al.*, 2011; R Development Core Team, n.d.)) Using the same package, the Chaol estimator was calculated in order to extrapolate the expected species richness in our study area (Colwell & Coddington, 1994). We employed sample-based species accumulation curves as an alternative to individual-based methods, which are known to over-estimate species richness when individuals are patchily distributed (Gotelli & Colwell, 2001). Species names conform to Pelham (2008).

Data were compared to faunal records from similar sites in the California Coast Range (Gates Canyon, 150-670 m a.s.l.) and the Sierra Nevada Range (Washington, 760 m a.s.l.) as well as the Central Valley floor (West Sacramento, 7 m a.s.l.; Fig. 2). Data from comparison sites were collected under similar conditions and during the same time period by A.M. Shapiro as part of an ongoing butterfly monitoring study (http://butterfly.ucdavis.edu).

Similarity Indices

A Chao-Soerensen index of similarity (C-S index) was calculated for the Sutter Buttes and each of the 3 comparison sites presence-absence data, implemented in R using the Fossil package (Vavrek, 2001). The Chao-Soerensen measure was selected in order to reduce the impact of under-sampling caused by traditional similarity indices (e.g. Jaccard index; Jaccard, 1912). Abundance data were not available for all surveys; therefore, presence/absence per site visit was used as a proxy for abundance (e.g., species "A" was seen 21 of 46 times at site 1, and 1 of 20 times at site 2).

In addition to calculating the C-S index for the Sutter Buttes and each comparison site we looked for shared unique species (SUS), defined as species found at the Sutter Buttes and only one of the three comparison sites during the study period.

After identifying species that were present at one or more of the comparison sites, but absent from the Sutter Buttes, we investigated host plant presence/absence for these missing species. We compared known host plant requirements for each missing butterfly species against Sutter Buttes floral records from Anderson (2004), the CalFlora database (2008), and our own observations.

The Papilionoidea species list from this survey was compared to the 1978 survey performed by Peoples (Table 1). Species absent from either survey were noted in order to assess the accuracy of Peoples' survey and to explore changes in butterfly faunal composition of the region since 1978.

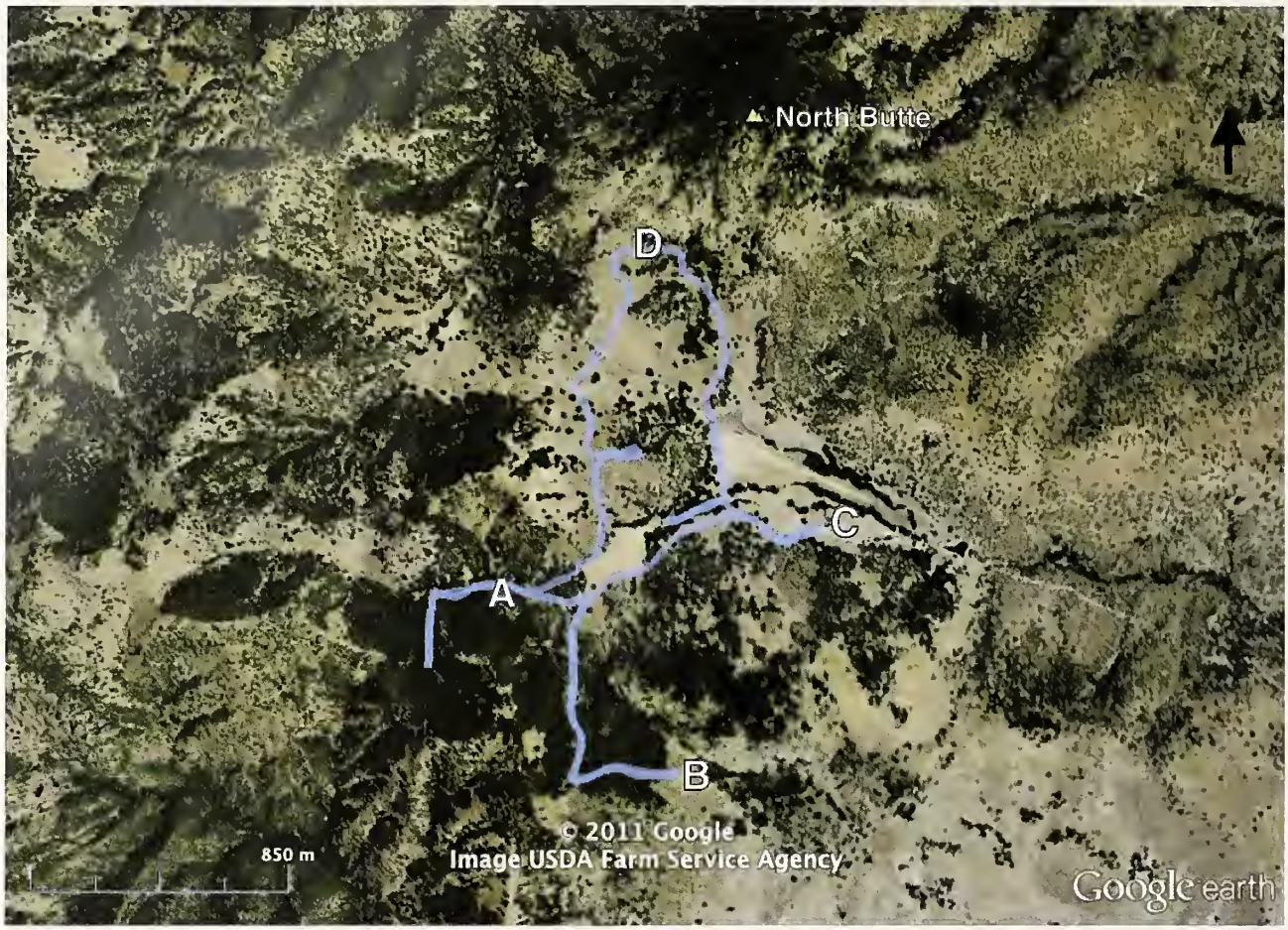

Figure 2. Survey transect with key sites labeled. **A**: Riparian corridor, **B**: High peak where hilltopping was prevalent, **C**: Dean's Ranch homestead, **D**: Chaparral/rocky outcroppings

Results

Species Presence/Absence

A total of 30 species were recorded in this survey at the Sutter Buttes study site, including 24 Papilionoidea species comprising four families and eight sub-families (Table 1). The species accumulation curve for Papilionoidea (Fig. 3) and the value of the Chaol estimator (27.6) suggest that there are several (~3-4) additional species present in the Sutter Buttes that were not recorded in the present survey. During the same time period, the comparison sites recorded 57 species (Washington), 52 species (Gates Canyon), and 25 species (West Sacramento), all Papilionoidea (http://butterfly.ucdavis.edu). A total of 39 species from 5 families and 9 sub-families were present at one or more of the comparison sites but absent in both surveys of the Sutter Buttes (Table 1). Two of those species, *Coenonympha tullia* Edwards 1871 and *Satyrium sylvinus* Boisduval 1852 were present at all three comparison sites, but absent from both surveys of the Sutter Buttes (Table 1). One species, *Aglais milberti* Godart 1819, was recorded at the Sutter Buttes, but not at any of the three comparison sites.

We found *Battus philenor* Linnaeus 1771 to be the most common butterfly species, outnumbering the next most common species (*Pieris rapae* Linnaeus 1758) by more than 3:1. This result was not shared by any of the 3 comparison sites. The remaining 23 Sutter Buttes species were generally found in lower numbers than at the comparison sites for which counts are available.

Butterflies were found throughout the transect in all different habitat types. Although a few species were restricted to only one or two habitats (e.g. *Anthocharis sara* Lucas 1852 was only found in one riparian area) most species were not associated with specific habitats.

Although we did not directly test for it, we did not generally observe phenological differences between species found at the Sutter Buttes and the three comparison sites. The lone exception was *B. philenor,* which was observed at the Sutter Buttes in large numbers until much later in the season (until August-September) than at any of the comparison sites (typically early July).

Site Similarity

Gates Canyon

Of the 52 Papilionoidea species (from 4 families, 11 subfamilies) recorded during the study period at Gates Canyon, 24 were also recorded at the Sutter Buttes. One species, *A. milberti,* was recorded at the Sutter Buttes but not at Gates Canyon. The calculated C-S index was 0.818. Gates Canyon was the only site to record an SUS with the current Sutter Buttes survey: *Papilio multicaudata* Kirby 1884. Two other species, *Lycaena xanthoides* Boisduval 1853 and *Euchloe ausonides* Lucas 1852, were SUS between Gates Canyon and Peoples' Sutter Buttes survey.

Washington

Twenty of the 57 Papilionoidea species (from 5 families, 13 subfamilies) recorded at Washington during the study period were also recorded at the Sutter Buttes. Four species, *A. milberti, Cupido comyntas* Godart 1824, *P. multicaudata,* and *Vanessa atalanta* Linnaeus 1758, were recorded at the Sutter Buttes but not at Washington. Washington had the lowest C-S index score of the 3 comparison sites at 0.642. There were no SUS between Washington and the current survey, although one was recorded from Peoples' survey (*Phyciodes pulchella* Boisduval 1852).

West Sacramento

West Sacramento shared 17 of the 24 Papilionoidea species (from 4 families, 10 subfamilies) recorded at the Sutter Buttes. An additional 8 species were recorded at West Sacramento but not at the Sutter Buttes. The C-S index between these two sites was the highest of the three comparison sites at 0.85.

Comparison to Peoples' (1978) survey

Peoples' survey of the Sutter Buttes found 33 Papilionoidea butterfly species (Table 1). There were 9 species found in Peoples' survey that were not recorded in our survey, and no additional species were found that had not already been recorded by Peoples (1978).

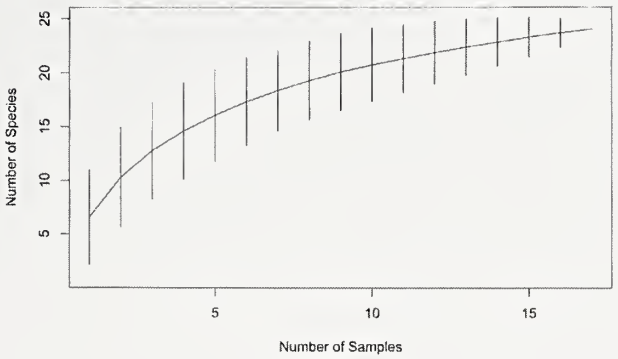

Figure 3. Mean species accumulation curve and its standard deviation based on random permutations of the data. A total of 24 Papilionoidea species were recorded. Because the number of species continues to increase with sampling effort, the curve suggests that additional species are present in the Sutter Buttes but were not recorded.

Host plant presence/absence

Of the species missing from the Sutter Buttes, only 9 could be explained by the co-absence of the larval host plants (Table 1). In the remaining 30 cases at least one host plant species has been recorded within the Sutter Buttes range.

Discussion

Of primary interest to our study was whether there is any indication of a dominant source region for the Sutter Buttes butterfly fauna. The Sutter Buttes are much younger than both the nearby Coast Range and Sierra Nevada Range, suggesting that any species found in either mountain range and in the Sutter Buttes but not in the Central Valley may have colonized the Sutter Buttes from one or both nearby ranges. Because certain species found in the Coast and Sierra Nevada ranges exhibit phenotypic differences, suggesting divergent lineages (e.g. *Euphydryas chalcedona* Doubleday 1847), their presence at the Sutter Buttes would immediately suggest a likely source. Likewise, a high number of SUS would suggest that either the Coast Range or Sierra Nevada Range contributed more to the butterfly fauna of the Sutter Buttes. However, our results indicate that the Sutter Buttes butterfly fauna most closely resembles that of the Central Valley floor despite bearing a stronger geologic and elevational similarity to the foothills of the Coast Range and the Sierra Nevada Range. Only one of the species recorded at the Sutter Buttes during the modern survey was identified as a

SUS (*P. multicaudata* was a SUS with Gates Canyon), and only one species is unique to the Sutter Buttes. That species, *Aglais milberti*, is a seasonal, altitudinal migrant that overwinters at low elevations and migrates to the Sierra Nevada mountains as adults. *A. milberti* was absent from the comparison sites during the timeframe of our survey, though it was last recorded at Gates Canyon in the mid 1990s, at West Sacramento in the late 1980s, at Washington in the early 1990s, as well as occasionally at additional study sites along the Central Valley floor. Based on these findings, there is no evidence to implicate either mountain range as a source population for any of the species at the Sutter Buttes.

Gates Canyon (Coast Range) and Washington (Sierra Nevada Range) shared 24 and 20 species with the Sutter Buttes, respectively. While both of these sites had a higher number of shared species than did the Central Valley site (West Sacramento at 17), both mountain sites had a much higher overall number of species than did the Sutter Buttes (52 and 57, respectively) or West Sacramento (25), resulting in a lower percentage of shared species and lower C-S index scores.

Several of the missing species at the Sutter Buttes are notable because of their otherwise weedy, ubiquitous nature. For example, the two species that were recorded at all three comparison sites (and missing at the Sutter Buttes) are widespread throughout the entire region. Likewise, three of the additional species found at West Sacramento but not at the Sutter Buttes (Table 1) support the suggestion that even several weedy species have been unable to colonize the Sutter Buttes. Based on the species accumulation curve (Fig 3) and the Chaol value of 27.6 species, we estimate that the present survey recorded approximately 87% of the butterfly species present in the Buttes. The remaining taxa are likely represented by rare, patchily distributed, or seasonally restricted species, making the absence of common and weedy species all the more conspicuous.

Since butterfly species distributions are closely linked to those of their host plants we investigated whether the depauperate butterfly fauna at the Sutter Buttes was linked to the co-absence of larval host plants. We found that of the 39 butterfly species present at one or more comparison sites but absent from the Sutter Buttes, only 9 could confidently be explained by host plant absence. In the other 30 cases, at least one potential host plant has been documented within the Sutter Buttes range (Anderson, 2004; Calflora Database, 2008).

Anderson (2004) points out that many plant species found in the Sutter Buttes are present at low abundance, a pattern we also observed during our survey. For example, while we recorded the presence of *Symphyotrichum subulatum* (Asteraceae), host plant for *Chlosyne palla* Boisduval 1852, it was restricted to one isolated site on the flank of the North Butte, and only a few plants were present. Furthermore, some other plant species were recorded predominantly in the property's riparian corridor; while this is certainly an important habitat, it makes up only a small portion of the total area at the Sutter Buttes. We observed a similar pattern for many butterfly host plants, suggesting that although the necessary plant species are present, their local densities may be inadequate for supporting a permanent butterfly population.

Furthermore, our broad treatment of host plant presence and absence included all acceptable host species, without focused attention to preferred host plants. There is evidence that some butterfly species that utilize multiple host plants may exhibit local specialization (e.g. *Euphydryas chalcedona* Doubleday 1847: Bowers 1986). While we recorded a large number of potential host plants at the Sutter Buttes, it is possible that the absence of preferred host plants has precluded butterfly immigrants from colonizing.

Inadequate abundance of adult nectar sources may also pose an impediment to butterfly colonization at the Sutter Buttes. There is evidence that nectar limitation may have a detrimental effect on butterfly population viability (Schultz & Dlugosch, 1999; Murphy *et al.*, 1984; Boggs, 2003). A number of regionally important nectar sources are absent from the Sutter Buttes. Most notable among them is the California Buckeye tree (*Aesculus glabra*), which is an important late-spring nectar source for a wide variety of butterfly species in this region. The flowers of this tree tend to bloom concurrently with peak spring butterfly flight, and a single tree is commonly visited by dozens of individuals of many species at a time. Other important nectar sources were either absent or present at low abundance, suggesting that nectar availability may be problematic at the Sutter Buttes. We observed anecdotal evidence for nectar limitation during our surveys. For example, we often found large numbers of *B. philenor* attempting to nectar on blackberry flowers (*Rubus* spp.). This is a very unusual behavior, as blackberries are bee-pollinated and likely do not produce substantial quantities of nectar for butterflies, suggesting inadequate local availability of preferred nectar sources.

Peoples' 1978 survey of the area around the South Butte (~3km from our transect site) recorded 33 Papilionoidea species, a surprisingly low number

relative to what might be expected for this region. While it is possible that Peoples' list represents an incomplete account of the butterfly species richness in the Buttes, we are unaware of any additional species being recorded at the Sutter Buttes in the time since her survey was conducted. Not only did our recent study fail to record additional species, we recorded 9 fewer species than did Peoples' survey, and estimated a total richness of ~5 fewer species (Chao1 = 27.6). Since the two studies were conducted on different properties within the Sutter Buttes it is possible that the species recorded by Peoples are not all present in our study area, or that they are present in our area but were not recorded. Alternatively, it is possible that some species have been extirpated from the Sutter Buttes in the ensuing years since Peoples' study. Similarly, although host plants have been recorded for many of the 'missing species' at the Sutter Buttes, we cannot assess the actuality of records that were not confirmed by our own observations, and it is possible that some plants have gone locally extinct from the Sutter Buttes since they were recorded. Although data were not available for comparison sites during the time corresponding to Peoples' survey (thus precluding the possibility of calculating similarity indices for Peoples' data) we interpret the results of the present survey as indicative of a possible decline in butterfly species richness at the Sutter Buttes in the time since Peoples' survey. While we cannot explain with certainty why butterflies in the Sutter Buttes might be in decline, Forister *et al.* (2010, 2011) have observed similar declines in butterfly species richness at sites in the Central Valley over the past 2-3 decades related to land-use change and climatic warming. Specifically, they found that 7 of the 9 species found in Peoples' survey but not the current survey were also declining at one or more Central Valley survey sites; the remaining two species, *Phyciodes pulchella* and *Papilio eurymedon* were not recorded at all at their Central Valley survey sites.

An additional impediment to butterfly colonization and persistence may be wildfire (Anderson, 2004). Most ecological communities in California are adapted to—and in fact benefit from—periodic wildfires. The grassland, oak woodland, and chaparral habitats of the Sutter Buttes are all examples of fire-adapted communities. It is likely that prior to European colonization, periodic fires burned portions of the Sutter Buttes without devastating effect. These fires are believed to have moved slowly through a mosaic habitat of burned and unburned areas, leaving butterfly and host plant refugia intact (Schoenherr, 1992). It is likely, however, that because of the Sutter Buttes' small size and relative isolation, fewer refugia are available in the Buttes, making butterflies and/or host plants more susceptible to large fires (or other environmental hazards) than species in the comparison sites. Furthermore, modern fire suppression policies have significantly altered the impact of fire in many ecosystems. Many of the fires that occur now often move through an area much more quickly, burn much hotter, and do more damage than the wildfires prior to European settlement (D'Antonio & Vitousek, 1992). A number of fires in modern times may have had an effect on wildlife in the Sutter Buttes. Several arsons burned large areas within the range in the 1960s (Anderson, 1983), and grass fires are always a possibility during drought years and throughout the summer dry season. During our study period a grass fire, believed to have originated at an electrical transformer, occurred near the South Butte. While we did not directly observe negative effects to the butterfly fauna from this fire we can not discount the possibility that host plants were destroyed or butterflies directly killed, including species of either that we did not record during our study.

While the Sutter Buttes are a truly remarkable mountain system in terms of their small size and isolation from other mountain ranges, there are several examples of larger but relatively isolated mountains across the world. Often deemed "sky islands," these mountains are characterized by physical separation from other, more continuous, mountain ranges, and by being surrounded by "seas" of lowlands consisting of dramatically different climates and environments. Sky islands are of great bigeographical and conservation significance as they often harbor relictual and/or endemic populations, and can provide important opportunities for vertical migration and environmental refugia in response to climate change (Forister *et al.*, 2010). Ideally, this study would compare our findings to butterfly surveys from other sky islands, though to our knowledge comprehensive surveys and comparative biogeographical investigations are often severely lacking in other isolated mountain systems, and the systems that are well-studied are not directly comparable to the Sutter Buttes. For example, the Madrean sky islands in southeastern Arizona are some of the best studied of the world's sky island ecosystems and the most geographically proximate to the Sutter Buttes. These mountains harbor well over 200 species of butterflies (Stewart, 2001; Bailowitz, 2007), a great deal more than our estimate for the Sutter Buttes, but are also much older, taller, larger, and less isolated than the Sutter Buttes (Drewes, 1972), making direct comparisons uninformative.

Faunal investigations of the world's sky islands are of the utmost importance considering the rapid loss of biodiversity worldwide, changes in regional climate, and land use change. We hope that this butterfly survey of the world's smallest mountain range will inspire similar surveys and biogeographical analyses of other sky islands to elucidate the patterns and processes that determine faunal composition in isolated ecosystems.

Conclusions

The present survey found the butterfly fauna of the Sutter Buttes to be surprisingly species-poor. In comparison to sites in the Central Valley, Coast Range, and Sierra Nevada Range, the composition of Sutter Buttes' butterfly fauna is most similar to that of the Central Valley floor, despite bearing more ecological and elevational resemblance to the mountain sites. Results from the present study were compared to a previous butterfly survey and suggest a decline in species richness in the Sutter Buttes since 1978, in keeping with regional declines observed by Forister *et al.* (2010, 2011). While we explored host plant availability, nectar availability, and fire history as possible explanations for the impoverished fauna at the Sutter Buttes, more detailed biogeographical, historical, and/or climatic analyses will be necessary to understand the ecology and biogeography of butterfly populations in this unique mountain range.

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Note

Notes on the immature stages of *Setabis* sp., a myrmecophagous riodinid butterfly (Lepidoptera: Riodinidae)

While visiting the state of Rondonia (Brazil), a curious butterfly larva was found in association with an unidentified ant species and subsequently reared to maturity on a diet of larvae and pupae of the Argentine ant, *Linepithema humile* (Mayr, 1868) (Hymenoptera: Formicidae). The adult, a female (Figs. 2 & 3), was identified as a member of the genus *Setabis* Westwood, 1851 (Lepidoptera: Riodinidae: tribe Nymphidiini), *fide* P.J. DeVries and D.J. Harvey, although without an associated male the specific taxon remains problematic. It appears to be at least near *S. lagus* (Cramer, 1777), and hereafter is referred to as *S.* cf. *lagus*. In addition to documenting the unusual diet of this insect in captivity, some morphological features of the last instar larva and pupa are reported.

The larva in question was given to the senior author by James Brock, who reported finding it "62 km S. Ariquemes, nr. Fazenda, Rancho Grande, 16 Nov. 1990" while investigating ant "carton shelters" on an unidentified shrub. The larva closely matched the dull mottled brown coloration of such shelters, which are often constructed over some riodinid larvae and honeydew-secreting homopterous insects (DeVries 1997; Ballmer pers. observ.). This larva was somewhat broad and dorso-ventrally compressed, while the prothorax was prolonged into a pair of short anterior extensions flanking and concealing the cranium; in habitus it closely resembled a larva of *S. lagus* illustrated by DeVries (1997), as well as larvae reported by Longino and Cover (2005) in association with *Pheidole biconstricta* Mayr (Hymenoptera: Formicidae).

The *S.* cf. *lagus* larva was initially confined within a 40 dram (147 ml) plastic vial with a leaf from the shrub on which it had been found. After three days, during which the larva crawled almost incessantly without feeding on the leaf, a few larvae of *L. humile* were added to the vial. The *S.* cf. *lagus* larva fed on the ant larvae immediately upon encountering them. It continued to feed on ant brood when it was subsequently transferred to a petri dish (55 mm diameter) to which additional *L. humile* larvae, pupae, and worker ants were added, along with a small leaf on which the larva settled. All *L. humile* worker and immature ants were obtained from the same wild colony. Pupation occurred on 4 Dec. 1990 and eclosion was about two weeks later (exact duration not recorded). Initial notes on external larval and pupal morphology were supplemented, following adult eclosion, by microscopic examination of the larval and pupal exuviae.

The worker ants moved all the ant immatures to the leaf and piled them near the stationary *Setabis* larva, which subsequently ate them. The *S.* cf. *lagus* larva moved from its stationary position on the leaf only when prodded by the observer, but subsequently returned to its former position (Fig. 1). The larva continued to feed on ant brood and approximately doubled in size over the next few days; it eventually pupated on the same leaf, after which all remaining adult and immature ants were removed. The *L. humile* workers did not display any specific attendance behavior toward the *S.* cf. *lagus* larva, aside from placing ant larvae in close proximity to it.

Although the diet of the *S.* cf. *lagus* larva was not observed in the wild, its demonstrated capacity to subsist on a diet of immature *L. humile* ants suggests that myrmecophagy, as a brood parasite, may be a normal behavior. *Setabis lagus* is reported to feed on immature homopterous insects (Kaye, 1921; DeVries *et al.*, 1992) and to pupate gregariously within "carton shelters" near the associated ant nest entrance (DeVries, 1997). The life histories of other *Setabis* species have not been reported.

Myrmecophilous riodinid larvae possess various specialized cuticular organs to mediate their ant associations. Three such specific ant organs have been reported on larvae of nymphidiine riodinids (DeVries, 1988): 1) vibratory papillae on the prothorax generate substrate-borne vibrations which may attract ants (DeVries, 1990; 1991); 2) anterior tentacle organs (ATOs) on the metathorax are

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believed to release semiochemicals which mimic ant pheromones; 3) tentacle nectary organs (TNOs) on abdominal segment eight (A8) secrete a fluid which ants imbibe. Some immature riodinids, in common with lycaenids, also possess tiny cuticular perforated cupola organs (PCOs = lenticles of some authors) and delicately branched dendritic setae, whose functions remain speculative, but are often concentrated on body regions at or near foci of ant attendance (Ballmer & Pratt, 1991). Of these organs only TNOs were observed while the larva was alive. Eversion of the TNOs was observed only once and very briefly (for approximately one second) when the larva was prodded, and did not appear to be accompanied by any fluid secretion; nor was any concurrent behavioral response to the everted TNOs observed among nearby worker ants. The ants did not display any greater attention to the *Setabis* larva than to the ant larvae; nor did they exhibit aggression toward it.

A pair of vibratory papillae is present on the larval exuvia beneath the anterior extensions of the prothorax and directly above the cranium. The larval dorsum is densely covered with short, broad setae, varying from conical to mushroom-like in appearance and with a minutely granular surface appearance (MS in Fig. 4). The short, narrow base of this type of seta is recessed below the surrounding cuticle and best seen in profile in dislodged setae. Similar setae present on the cranium are most apparent on the frons and genae, gradually reduced in size and density dorsally, and virtually absent on the epicranium. The color of these setae (beige, brown, or black), their low reflectance, and their distribution pattern impart a dull mottled appearance similar to that of an ant "carton shelter." Setae of similar structure were illustrated by DeVries (1988) on the prothorax of *Thisbe irenea* (Stoll, 1780) larvae. More elongate, tapered tactile setae (Fig. 4) occur peripherally along the lateral fold, anterior margin of the prothorax, and lower portions of the cranium, especially near the oral margin.

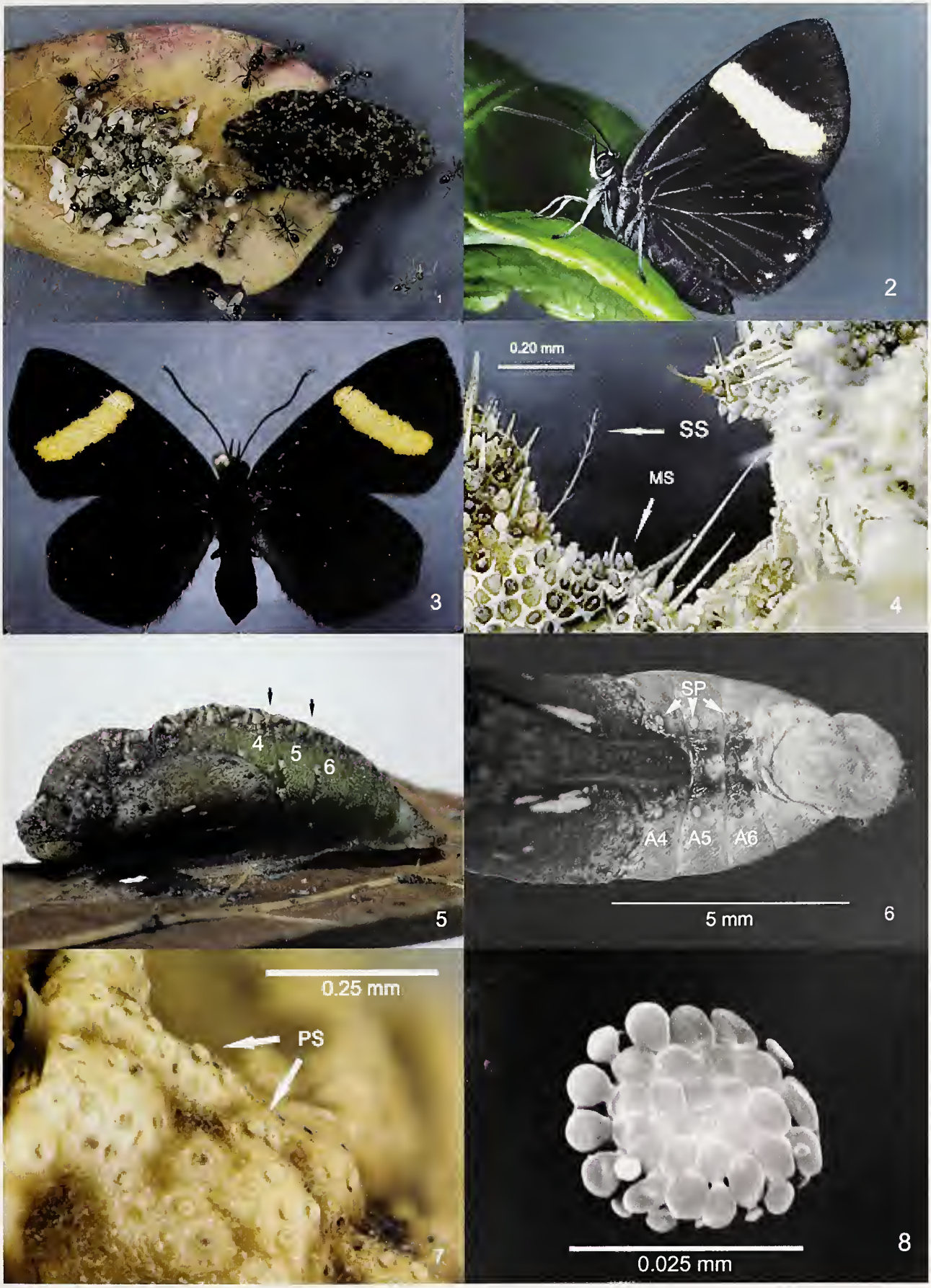
A pair of slender tonosensillae ("sensory" setae *sensu* Ballmer & Pratt, 1988; also see discussion in Ballmer & Wright, 2008) is present anteriorly on the prothorax (SS in Fig. 4). These setae are easily distinguished from nearby tactile setae by their more slender shaft, somewhat greater length, recessed basal attachment, and slender lateral filaments. Structurally similar setae were reported for *Juditha caucana* larvae by Hall & Harvey (2002).

The *S.* cf. *lagus* pupa is roughly sculptured and rugose (Figs. 5 & 7), somewhat resembling that of *Thisbe irenea* (DeVries, 1997), generally mottled in earth-tones, but with olive green abdominal pleura. A silk girdle is lacking so that attachment to a silk pad on the substrate is only by cremastral hooks. The venter is somewhat arched and the fused segments A8-10 are expanded ventro-laterally into a broad, nearly circular structure. The central venter of the fused terminal segments is recessed, forming an inverted bowl structure in which only the rim contacts the substrate (Fig. 6). Although numerous cremastral hooks are distributed over the ventral surface of the fused terminal segments A8-10, they are absent from the recessed central portion and only those near the periphery engage strands of the silken pad.

The dorsal and lateral surfaces of the pupa appear macroscopically glabrous, but have scattered clusters of tiny parasol-like setae (PS in Fig. 7). The recessed bases of these setae are concealed by their flattened disk-like distal portions, which bear bar stool-shaped protuberances (Fig. 8). Somewhat similar (i.e. parasol-shaped) setae are present on pupae of some other Riodininae, including *Lyropteryx* Westwood, 1851 sp., *Melanis lycea* Hübner, 1823, and *Rhetus periander* (Cramer, 1777) (Ballmer, pers. observ.), and *Aricoris erostratus* (Westwood, 1851), as illustrated by Schremmer (1978). Although simple elongate tactile setae are not present, patches of about 20-30 slender, apically bifurcate setae (some perhaps trifurcate) are present in localized patches anterior to the T1 spiracles and subventrally on A5 admixed with PCOs (Fig. 9). The slender branched structure of these setae is similar to dendritic setae found on larvae and pupae of some lycaenids (see Ballmer & Pratt, 1988) and riodinids such as *Eurybia* [Illiger], 1807 (Ballmer & Pratt, 1991).

The arrangement of abdominal spiracles on the pupa is remarkable. Spiracles are not visible on A1, A3, A7, and A8, while the A2 spiracle is prominently visible laterally, just above the point of contact of that segment with the wing case. Spiracles on A4-6 are present ventrally and were observed only after the pupal exuvia was detached from its silken pad (Fig. 6). The

Figures 1-8. (Opposite page). *Setabis* cf. *lagus*. 1. *Setabis* cf. *lagus* larva with *Linepithema humile* adults and immatures. **2.** Adult *Setabis* cf. *lagus* female. **3.** Female *Setabis* cf. *lagus*, dorsum. **4.** T-1 sensory (SS) and mushroom (MS) setae on larval exuvia. **5.** *Setabis* cf. *lagus* pupa indicating sites of intersegmental stridulatory organs. **6.** Pupal venter indicating spiracles on A4-6 and possible resonating chamber on fused A8-10. **7.** Parasol setae (PS) on A6 dorsum of pupal exuvia. **8.** SEM image of pupal parasol seta.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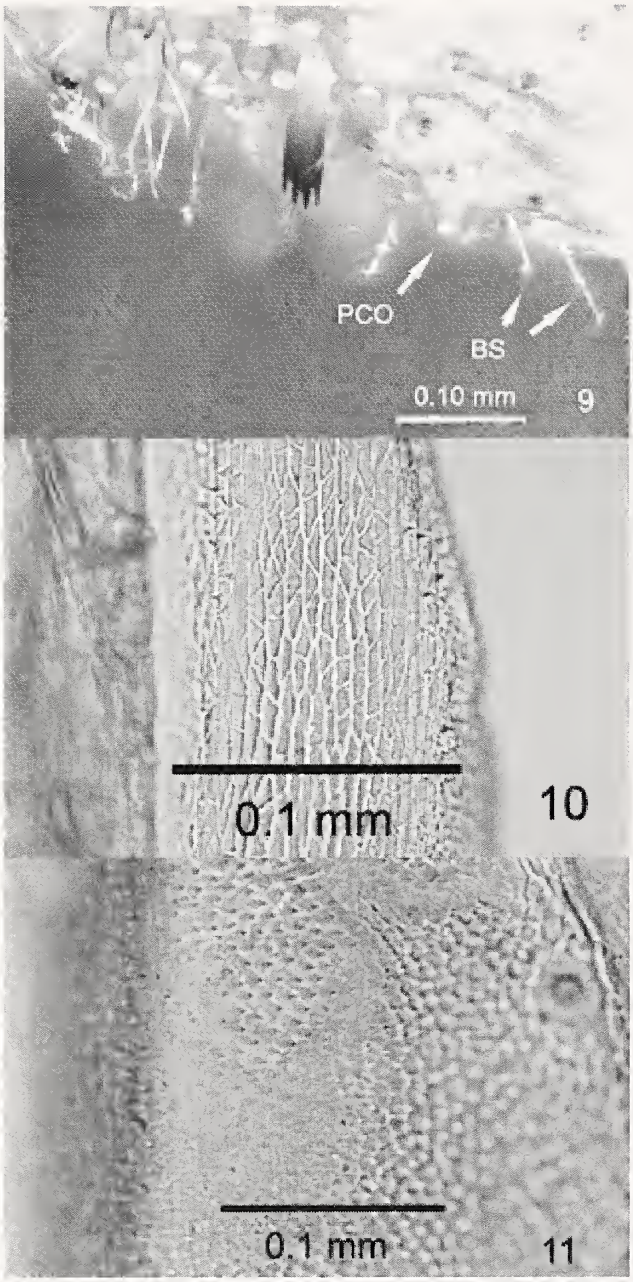

Figures 9-11. ***Setabis* cf. *lagus*. 9.** Bifurcate setae (BS) & PCOs on pupal venter, A5. **10.** Stridulatory plate in intersegmental cleft, pupal dorsum, A4. **11.** Stridulatory file in intersegmental cleft, pupal dorsum, A5.

arched venter of the pupa in this region may facilitate air exchange with the spiracles. The absence of spiracles on pupal abdominal segments 1 and 3 is typical of many (perhaps most) genera of Riodininae, but not the Euselasiinae and Hamearinae (Hall *et al.*, 2004). The ventral placement of spiracles on A4-6 has not been reported in other genera.

Inspection of prominent dorsal intersegmental clefts at the junctions of A4/5 and A5/6 (Fig. 5) revealed a stridulatory plate consisting of a honeycomb of polygonal ridges near the posterior margin of the cephalad segment (Fig. 10) and a facing file of raised teeth near the anterior margin of the caudad segment (Fig. 11) of each segment pair, similar to those reported for various lycaenids and riodinids (Downey, 1966; Downey & Allyn, 1973 and 1978; Nishida, 2010; see also Álvarez *et al.*, 2013). Further inspection revealed that the stridulatory organs continue ventrally and encircle the relevant segments, as reported for some other riodinids (Downey, 1966). The inverted bowl structure of fused segments A8-10 may serve as a resonating chamber to amplify stridulations.

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Spatial distribution and habitat assessment of *Panoquina errans* (Lepidoptera: Hesperiidae) in San Diego County, California

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Abstract. *Panoquina errans* (Skinner, 1892), commonly known as the wandering skipper, is restricted to a narrow band of disjunct salt marsh habitat extending along the west coast of North America from Santa Barbara Co., California to the southern tip of Baja California, Mexico. A determination by the U.S. Fish and Wildlife Service of whether it represents an endangered or threatened species could not be made owing to a paucity of information on its biological vulnerability and threat. Based on a three-year survey (2010-2012) in San Diego Co., California, the species was observed in nine coastal lagoons and a coastal bluff. At all sites within the study area there was a significant correlation between the maximum annual observations of *P. errans* and the total area occupied by the larval host plant *Distichlis spicata* (Poaceae). The primary habitat for *P. errans* is coastal lagoons and coastal bluffs (100% of observations); elevations less than 5 m above mean sea level (98% of observations); within 25 m of patches of *D. spicata* over 1 m^2 (75% of observations); and containing *Frankenia*, *Cakile*, or *Heliotropium* (95% of observations).

Key Words: threatened and endangered species, salt marsh, California, Mexico, conservation.

Introduction

Panoquina errans (Skinner, 1892) occurs along the West Coast of North America from Santa Barbara County, California to the southern tip of Baja California Sur, Mexico (MacNeill, 1962; Donahue, 1975; Wells *et al.*, 1983; Faulkner & Klein, 2012). However, this wide distribution conceals the fact that it is restricted to a narrow band of disjunct coastal lagoon habitat that has diminished considerably over the last century. Although *P. errans* was considered a candidate for listing as endangered or threatened by the U.S. Fish and Wildlife Service (FWS) in 1994, it was determined that "persuasive data on biological vulnerability and threat are not currently available to support proposed rules" (FWS 1994).

Within San Diego County, populations appear to be small and stable throughout the coastal marshes from Buena Vista Lagoon south to the U.S./Mexico border (Faulkner & Klein, 2012). The primary threat to the species has been the filling and/or dredging of coastal wetlands (Wells *et al.*, 1983), which is estimated to have been reduced by almost 88% in San Diego County (Oberbauer & Vanderwier, 1991; Zedler *et al.*, 2001). In addition to historical habitat loss, the threat of sea level rise, estimated to be between 12 and 61 cm by 2050 (National Academy of Science, 2012), may pose a significant future threat to this species (Reed, 1995; Zedler, 1996).

Little is known about the distribution, ecology, or population dynamics of the wandering skipper (FWS, 2008). In order to more effectively manage and conserve this species, I conducted investigations to define the current distribution of *P. errans* in San Diego County estuaries; define the key characteristics of occupied *P. errans* habitat at various spatial scales; and identify the commonly used nectar sources. These features were identified as critical uncertainties for the management of the species in San Diego County (FWS, 2008).

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Methods

Study Area. The study area included ten sites (nine coastal lagoons and one coastal bluff) in San Diego, California (Fig. 1). The region has a Mediterranean

climate characterized by warm to hot, dry summers and mild to cool, wet winters (Dallman, 1998). San Diego's remaining coastal lagoons are small and discrete, confined to narrow river valleys separated by coastal hills (Zedler, 1982). The vegetation in these lagoons follows a vertical zonation from tidal creeks, through an intertidal zone of low to high marsh comprised of halophytic vegetation, to a transitional area of terrestrial vegetation (Penning & Callaway, 1992). Zedler (1982) characterizes the marshes as having low species richness, but showing a "wide variation in vegetation structure and functions from marsh to marsh, as well as within individual wetlands." The *P. errans* larval hostplant, *Distichlis spicata* (commonly referred to as saltgrass) (Well *et al.*, 1983; Brown, 1991), is a non-obligatory halophyte that can survive where the water table varies between 15 cm below and 5 cm above soil surface (Hansen *et al.*, 1976).

The nine lagoons included in this study run generally east to west from the ocean, have a range of hydrologic connectivity to the ocean, and are surrounded by a range of different land uses. These lagoons span the western edge of San Diego County, and were selected to confirm the occurrence of *P. errans* from historical reports and to survey appropriate habitat lacking information on the occurrence of the species. Access was limited to areas open to the public or where land managers gave permission for surveys to be conducted. Coastal bluffs were initially excluded from the study due to the perception of the low probability of occurrence from past literature, but an opportunistic sighting of an individual of *P. errans* on a coastal bluff suggested I should expand the survey area; one coastal bluff was included in the last year of the survey.

Field Surveys Protocols. The study was conducted from 2010-2012. During the first year, five lagoons (Table 1) were selected to determine the presence/absence of *P. errans* and determine its general distribution within the lagoons. The study began under the premise that habitat requirements of the wandering skipper were unknown (FWS, 2008), and therefore exploratory surveys were conducted in August and September of 2010. Survey methods generally followed those recommended by the FWS (2008) which state that they "should be conducted between 1000-1500 hours, when temperatures are between 65–90°F (18–32°C) and wind speed less than 10 mph (16 km/h)."

Surveys were conducted by two investigators. Each carried field binoculars, a camera, a net, and field guides (Emmel & Emmel, 1973; Bryant, 2010). The "checklisting" butterfly survey method described by Royer, Austin & Newton (1988) was used, walking

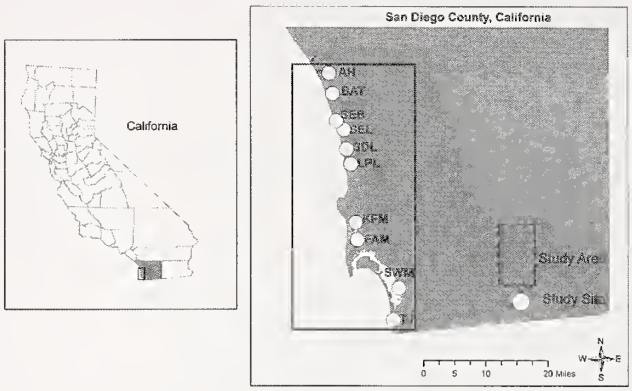

Figure 1. Location map of study area and study sites

a meandering transect through the lagoon until an individual skipper was observed. When an individual was discovered, the investigators walked in expanding concentric circles around the individual until no additional individuals were observed because progress was hindered by impassable tidal channels or terrestrial vegetation. This method allowed us to maximize the observation of individuals.

The second investigator was staggered approximately 10 m behind and 10 m to the left or right of the first. No active pursuit was made during the survey, and only individuals that were positively identified were recorded by the first investigator using a Trimble GeoExplore submeter accurate global positioning system (GPS). Observations from the second investigator were not counted unless subsequently observed by the first. The second investigator also kept track of individuals already counted that flew ahead of the first; these individuals were not recorded. The low height of the salt marsh vegetation allowed for excellent visibility, but tidal channels made some areas inaccessible. The average speed of the investigators was 12.1 m/min as tracked through the "routes" functions of the GPS. The results of the first year survey were downloaded into a Geographical Information System (GIS) running ArcGIS 10.1 to identify directional distribution polygons of skipper observations in the study area by generating one and two standard deviation ellipses around the observations (Mitchell, 2005). The average wind speed, humidity, temperature, and percent cloud cover were recorded prior to each survey using a Kestrel 3000 pocket weather meter. The elevation was obtained for each observation of *P. errans* from Lidar data from the National Oceanic and Atmospheric Administration (2012) with a minimum vertical accuracy of 9 cm.

Table 1. Survey information on study sites, year sampled, environmental conditions, detection dates, and extent of *Distichlis spicata* size mapped in the study site.

Study sites	Label	# of survey locations	Years sampled	Area (ha)	Total skippers observed	Max annual skippers	Avg wind (kph)	Avg hum %	Avg temp C_t	Avg % cloud cover	Earliest detection date	Latest detection date	Sum of *Distichlis spicata* (m^2)
Agua Hendonia	AH	1	2011	0.6	11	11	3.6	68.4	22.4	0.0	8/31/2011	8/31/2011	175
Batiquitos	BAT	1	2012	1.44	43	43	3.4	68.1	21.0	30.0	7/2/2012	7/2/2012	219
Famosa Slough	FAM	2	2010-2011	1.86	167	108	4.6	72.9	22.8	24.0	7/29/2011	8/31/2011	1525
Kendall-Frost Marsh	KFM	2	2010, 2011	4.33	2	2	3.0	73.7	24.4	22.0	8/27/2010	8/27/2010	10
Los Penasquitos Lagoon	LPL	3	2010-2012	19.1	121	86	4.6	68.5	25.5	22.5	7/14/2011	8/17/2010	1202
San Dieguito	SDL	2	2011	1.85	18	18	9.5	81.0	20.3	15.0	8/18/2011	8/18/2011	168
San Elijo	SEL	3	2010-2011	1.89	114	38	5.6	74.4	22.1	20.7	7/22/2011	8/31/2011	784
San Elijo Beach	SEB	1	2012	0.31	33	33	1.8	64.9	29.8	0.0	8/8/2012	8/8/2012	374
Sweetwater Marsh	SWM	3	2010-2011	4.41	29	15	9.7	72.3	23.5	0.0	8/3/2011	9/3/2010	780
Tijuana Estuary	TJ	3	2012	6.41	8	8	10.9	79.5	22.0	0.0	7/9/2012	8/7/2012	202
Total:	**10**	**21**		**42.2**	**546**								**5439**
Average:							**5.7**	**72.4**	**23.4**	**13.4**			**543.9**

During the second year, surveys for adults were conducted from 14 July 2011 through 31 August 2011 inside the two standard deviation ellipses generated from year 1. The surveyors utilized a Pollard walk methodology (Pollard, 1977; Pollard & Yates, 1993) within the ellipses along defined transect routes to maximize coverage within ellipses. Observed adults of *P. errans* and the plant species upon which they were nectaring and/or resting were recorded using GPS. The data were imported into GIS and was used to revise the standard deviation ellipses generated from the year 1 observations. For each revised ellipse, the mean center of the ellipse was calculated and a centerline bisecting the one standard deviation ellipse was established.

An assessment of the vegetation was conducted using systematic random sampling. Along the center line within the one standard deviation ellipse, a transect was laid out using a 100-meter field measuring tape. A point was selected at random within each 5-meter segment of the transect. A perpendicular transect was placed at the point extending between 10-25 m on each side of the transect and a point count was made for each plant species using a point intercept method (Elizinga *et al.*, 1998). Plant taxonomy followed the Jepson Manual (Baldwin *et al.*, 2012) as updated by the Jepson Flora Project (2012). The result was a grid of point counts of plant species within the one standard deviation ellipses that was used to characterize the area occupied by the adult wandering skippers.

In addition to transects, a series of plots based measurements were made to determine the percentage of plant species coverage (Elizinga *et al.*, 1998). A series of computer-generated random points were selected within the 1 standard deviation ellipses. A 1-m^2 plot was placed over a computer-generated random point, and abundance of plant species and percent cover estimates were collected using visual estimation. Two additional 1-m^2 plots were placed at 5 m from the center of the original plot at angles chosen at random between 1-180 degrees and 181–360 degrees of magnetic north. The result was an assessment area with a diameter of 10 m that included all three plots. If a skipper was observed at any time in the assessment area, the plots were considered occupied. The process was repeated until at least three groups of plots were measured that were recorded as occupied and three as unoccupied. While the transect data provides more information about each study site, the plot data allows for characterization of the vegetative cover of occupied and unoccupied areas within the study site. In addition to the transect and plot based vegetation surveys, a census of *Distichlis spicata* polygons greater than 1 m^2 with at least a 50% relative density (determined by visual estimation) was conducted within the one standard deviation ellipses that was used to characterize the area occupied by the adult wandering skippers.

In August 2011, two new lagoons were surveyed following the methods described above. While line transects were not conducted, plot assessments of the habitat were obtained and a census of *D. spicata* was done as described above.

The final year of surveys (2012) focused on expansion of the surveys, plot-based habitat assessments, and identification of *D. spicata* polygons into two additional lagoons that appeared to have suitable habitat and a third location where an opportunistic observation of a skipper occurred on a coastal bluff.

Data Analysis. A correlation analyses was conducted between the number of *P. errans* observed and the abundance of *D. spicata*, distance of individual to nearest *D. spicata* patch and open water, and elevation for each study site. Correlation coefficients and probabilities were determined using Spearman's rank correlation coefficient (r_s) due to the non-normality of some of the data (e.g., distance to *D. spicata* patch and open water appears to follow a Poisson distribution).

Multivariate analysis of the assemblage structure of plant species from transect data were conducted using non-metric multidimensional scaling (NMDS) (Mather, 1976) and a hierarchical, agglomerative cluster analysis using Primer-6 software (Clarke & Gorley, 2006) selecting group average methodology. Soerensen's distance measure of dissimilarity was used in both NMDS and cluster analysis. NMDS is a non-parametric approach to represent relationships between objects in multidimensional space using a matrix of ranked order of dissimilarity between objects (Quinn & Keough, 2002). Soerensen distance (also known as Bray-Curtis index when used for abundance data: McCune *et al.*, 2002) was selected because it ignores variables that have zeros for both objects; shared absences common in community studies do not affect the results (Field *et al.*, 1982; Faith *et al.*, 1987).

Transect data were standardized so that each site had a maximum total abundance of one (i.e., relative frequency to standardize for various study site sizes) and rare species, those that together contributed less than 10% of the overall abundance at a study site, were excluded to reduce noise in the data set without losing information (McCune *et al.*, 2002). An initial random start was selected in the ordination software to evaluate up to six axes to discern the usefulness of each. Stress values less than 10% were considered to represent a good ordination with no real risk of drawing false inferences as suggested by Clarke (1993). A similarity profile (SIMPROF) analysis was performed on the results of the cluster analysis to test for significance in structure of the clustered data using a significance level of 5% and Bray-Curtis similarity.

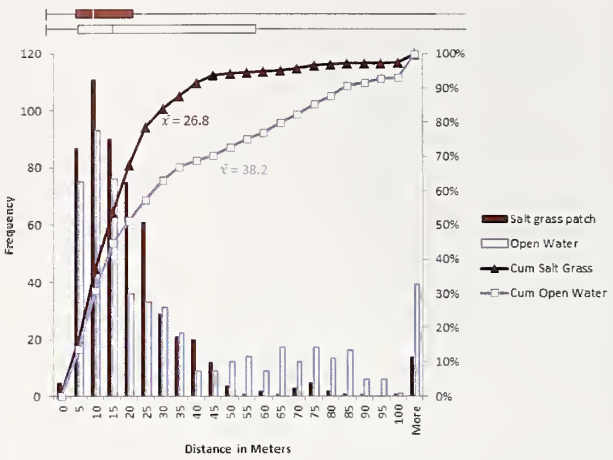

Figure 2. Frequency of *P.errans* observations compared to location of patches of *Distichlis spicata* (saltgrass) and open water. Bars represent frequencies in 5 m distance bins and line represents the cumulative number of skippers. Average is shown for all observed skippers (N= 546) from 2010-2012.

Data collected from plots were run through the same analysis structure (NDMS and cluster analysis) described above for the transect data. In addition, individual plots were grouped as occupied or unoccupied by skippers for the purposes of analysis. One-way Analysis of Similarity (ANOSIM) (Clarke 1993) was performed on the plots using the Primer-6 software to test for differences between group and community samples using a randomization test. Pairwise comparisons were summarized using global R (Clarke, 1993), a statistical measure that compares between-group to within-group dissimilarities. Monte Carlo permutation tests were used to determine statistical significance.

Data collected on the use of individual plants for nectaring and/or resting by skippers were evaluated among study sites and against the availability of that plant species at each study site using a two-tailed, paired Student's t-test (n = 10) after the data was arcsine transformed to account for utilization of relative frequency data.

Results

Skipper Distribution. *Panoquina errans* was present in each of the nine lagoons surveyed and was also found on a coastal bluff (Table 1). Although well distributed throughout the study area, within each lagoon skippers were restricted to discrete areas of the study sites. Skippers were detected at all study sites during every

survey resulting in a high probability of detection, except for Los Penasquitos Lagoon (LPL3) were no skippers were observed during three visits to the site.

Across all study sites, skippers occurred on average within 26.8 m of *Distichlis spicata* patches and 38.2 m from the edge of open water (Fig. 2). A boxplot of distance of observed skippers to nearest recorded *D. spicata* patch indicates that over 75% (third quartile) of the observed skippers are within 25 m from *D. spicata* patches (Fig. 2). Distance to open water is more variable with the third quartile occurring at 55 m. The average elevation was 3.0 m above mean sea level (MSL) with a high of 6.5 m, a low of 1.7 m and a standard deviation of 0.67 m.

There was a strong (r_s = 0.806) and significant (p = 0.005) correlation between the maximum annual count of observed wandering skippers and the total area of *Distichlis spicata* in the study site (Table 2). Correlations between the count of skippers observed in the study sites and the average distance to nearest *D. spicata* patches, open water, and elevation were all negative, but weak (r_s = -0.200,-0.127 and -0.491, respectively) and not significant (p = 0.527, 0.687, and 0.147, respectively; Table 2).

Species composition and abundance. A total of 4868 individuals representing 66 plant species were recorded from transects (n = 4250/ 60 species) and plots (n = 617/ 47 species). Overall three species were responsible for 63% of the abundance in the study area: *Salicornia pacifica* (25.2%), *Frankenia salina* (23.3%) and *Distichlis spicata* (14.5%); however, variation did occur among study sites, especially at SEB which is discussed later. The majority of the plant species recorded (n = 51) contributed less than 10% to the overall abundance in the study sites. After standardizing and eliminating rare species, a total of 26 plant species of the 60 species remained from the transect data (Table 3). NMDS ordination recommended a 2-dimensional solution with a final stress of 5.3% after 35 iterations (Fig. 3a). Axis 1 and 2 cumulatively accounted for 93.2% of the variation. An analysis of outliers indicated that SEB was 2.14 standard deviations away from the average Soerensen distance of the other sites. All other sites were within 1 standard deviation. This result is not surprising given that SEB is a coastal bluff, whereas the other sites are within coastal lagoons. SEB does contain a significant abundance of *D. spicata* (32.6%), but has no *Frankenia* or *Salicornia*. In addition, SEB has a high percentage of non-native plant species (59.5%). The cluster analysis of transect data showed a weak structure with SEB being the only significantly distinct cluster (p = 0.04) compared to the other study sites); there was no significant difference between the lagoon study sites.

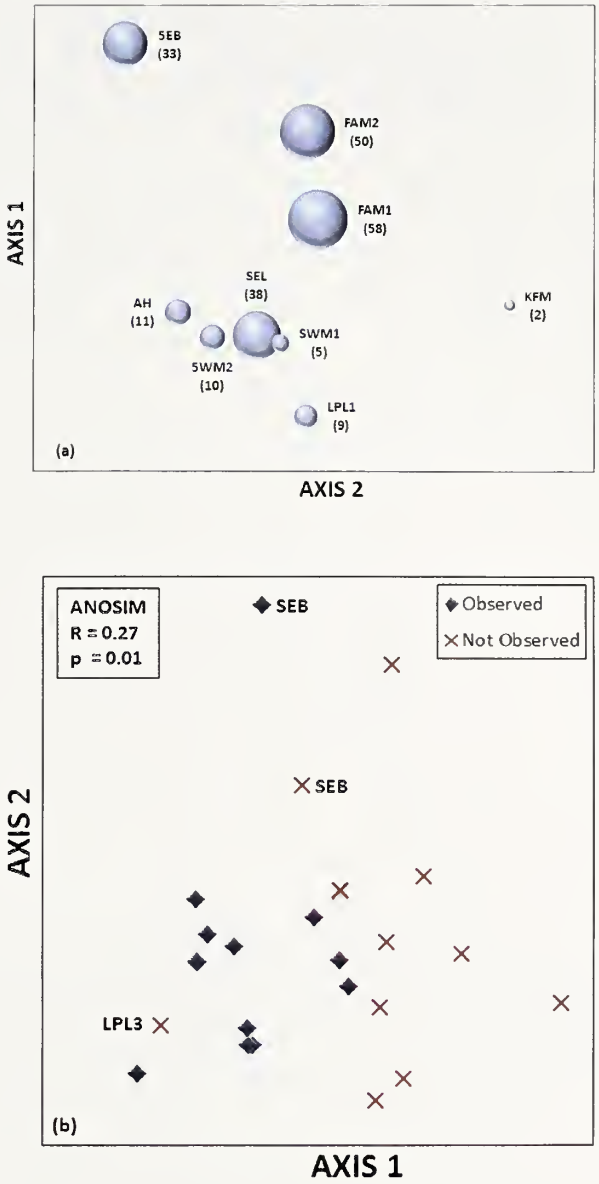

Figure 3. Results of non-metric multidimensional scaling (NMDS) on vegetation data collected from transects and plots. (a) NMDS labeled by study site on transect data. Area of symbols represents size of maximum annual observations (shown in parentheses) next to study site label. (b) NMDS by study sites on plot data. ANOSIM score of plots where skippers were observed and not observed displayed with probability of occurring by chance alone.

Data collected from the plots were run through NMDS to evaluate the assemblage structure of plant species in plots that contained adult skippers and those that did not. NMDS ordination on the plot data recommended a 3-dimensional solution with a final stress of 8.2% after 202 iterations (Fig. 3b). Axis 1, 2 and 3 cumulatively accounted for 92.7% of the variation. Figure 3b shows the ordination

Table 2. Maximum of annual skippers observed by study site compared to environmental covariates. Results of Spearman's rank correlation regression analysis and probably of occurring by chance alone are shown. Bold italics indicate significance at (α = 0.05).

Study Site	Max annual skippers observed	Sum *Distichlis spicata* (m^2)	Average distance to *Distichlis spicata* (m^2)	Average distance to open water (m^2)	Average elevation m above MSL
AH	11	175.0	91.1	8.1	3.0
BAT	43	219.0	16.6	13.1	2.6
FAM	108	1525.0	10.7	19.8	2.5
KFM	2	10.0	3.8	15.6	2.9
LPL	86	1202.0	56.0	24.0	2.8
SDL	18	168.0	15.9	22.9	3.8
SEL	38	784.0	36.7	14.0	3.9
SEB	33	374.0	77.3	8.1	3.0
SWM	15	780.0	104.1	133.5	2.9
TJ	8	202.0	68.7	202.1	3.1
r_s		***0.806***	-0.200	-0.127	-0.491
p		***0.005***	0.527	0.687	0.147

with plots occupied by skippers and plots not occupied by each study site. An ANOSIM indicated a significant difference between those plots identified as unoccupied and occupied (global $R = 0.27$; $p = 0.01$). The cluster analysis resulted in a clear distinction between the coastal bluff site, San Elijo Beach (SEB), from the lagoon plots ($p = 0.03$), but was not distinct ($p = 0.07$) from areas within SEB identified as occupied and not occupied. Plots located in coastal lagoons identified as not occupied by skippers were distinct ($p = 0.001$) from those that are occupied. Los Penasquitos 3 (LPL 3) was the outlier ($p = 0.009$), clustering distinct from all other groups, but more closely related to plots identified as occupied even though no skippers were observed.

Nectaring and Resting. Of the 546 observations of wandering skipper, nectaring or resting was observed on only eight plant species, and 97.6% of all sightings occurred on just three species (*Frankenia salina, Distichlis spicata* and *Cakile maritima*; Table 4). While 3.5% of the observations were on *D. spicata*, this is assumed to be "resting" because grass species have no nectar. In the lagoons, *Frankenia* by far was the most commonly used species. Along the coastal bluff site at SEB, *Frankenia* was absent and the skippers were observed on *Cakile* and *Heliotropium*. Among all study sites, *Frankenia* was used significantly more than expected by chance when compared to abundance of other plant species within the study area ($p < 0.001$), whereas *Distichlis spicata* and *Salicornia* were significantly under-used compared to their abundance in the study sites ($p = 0.019$ and $p = 0.0009$, respectively). The other five plant species upon which wandering skippers were observed were not utilized significantly different ($p >> 0.05$) compared to their abundance within the study sites (Table 4).

SIMPER Analysis. When the plots were grouped as occupied or unoccupied by skippers, the overall average vegetation dissimilarity among the groups was 68.18%. *Frankenia* was three times as abundant in occupied sites as unoccupied, while *Salicornia pacifica* was twice as likely in unoccupied sites as occupied. *Distichlis spicata* was equally likely to occur in either group. The abundance of these three species alone contributed 92.63% of the similarity between study sites identified as occupied and 87.24% between study sites identified as unoccupied. Los Penasquitos Lagoon 3 (LPL3) was kept with the unoccupied group for this analysis because it represents a single albeit unique sample.

Because SEB was a coastal bluff site that lacked lagoon vegetation, it was separated into a third group. Separating SEB, did not significantly change the average dissimilarity between lagoon groups identified as occupied and unoccupied (63.73%), but the average dissimilarity of SEB was greater than 92% compared to group 1 (occupied) and 93% compared to group 2 (unoccupied). *Cakile maritima, Distichlis spicata* and *Carpobrotus edulis* were the three key contributors to the coastal bluff site which lacked *Frankenia salina* and *Salicornia pacifica*. Among all three groups of plots, *Distichlis spicata* was always present and contributed between 10 to 14% of average abundance.

Discussion

Distribution. *Panoquina errans* was found in each of the lagoons and the one coastal bluff surveyed. Within all study sites, however, skippers were clumped in their distributions. The abundance of skippers varied among sites independently of the size of the sites. A low of two skippers were observed at the 4.6 ha Kendal Frost Marsh (KFM), and a high of over >150 individuals were recorded at the 1.86 ha Famosa Slough (FAM).

The nine lagoons likely cover the full range of potential habitat in San Diego County. Two lagoons, San Luis Rey and Buena Vista, were excluded from the study due to their very limited tidal habitat.

The chance observation and subsequent survey of a population of skippers along a coastal bluff maybe the most interesting aspect of this study, and corroborates an observation by Orsak (1977) of a skipper in heavily disturbed coastal bluffs. Subsequent reconnaissance surveys in the summer of 2013 (not included in this analysis) have confirmed additional observations along coastal bluffs in Carlsbad, California (between study sites AH and BAT), and along Del Mar to the north of LPL. Preliminary genetic data suggests that the coastal bluffs may serve to connect the coastal lagoon populations and help maintain gene flow (Daniel Marschalek, pers. comm.).

This study could not confirm a previous hypothesis (Zedler, 1982) that the largest population in the United States exists at the Tijuana Estuary (TJ). I found skippers in multiple locations in TJ at low abundance; however, the largest area of *Distichlis spicata* observed in the entire study area (over 1.5 ha) occurs along the northern arm of Oneonta Slough. This area was restricted from surveys due to the breeding season of endangered birds, but holds promise for an abundant number of wandering skippers.

Key Characteristics of Habitat. All survey sites contained *Distichlis spicata*, but they were not necessarily dominated by *D. spicata* (average 14% cover with a range from 0–30%). More significant was the size of the *D. spicata* patches within study sites. For example, small areas of *D. spicata* (10 m^2) were observed at KFM, a site that yielded only two sightings of *P. errans*. In contrast, FAM had 1525 m^2 of *D. spicata* where 108 skippers were observed. Also of note was that 75% of skipper observations were recorded within 25 m of a *D. spicata* patch greater than 1 m^2.

The habitat characteristics within the lagoon and along the coastal bluffs are distinct as discussed in the results of the cluster analysis and should be separated for identification of key characteristics that make up the habitat. The lagoon sites where skippers were observed contain an abundance of native plant species and are not significantly different in their composition. Across all study sites *Frankenia, Salicornia* and *Distichlis spicata* dominate the lagoon study sites with an abundance of 63% of the total plant cover recorded. The majority of species richness in the lagoon sites (51 species) comprises less than 10% of the abundance. Among study sites, 46 species (70%) occurred at less than 4 of the 9 lagoon study sites. This is consistent with Zedler's observation (1982) of the marshes lacking species richness, but showing a wide variation in vegetation from marsh to marsh.

Of interest is the negative association of skippers with *Salicornia*. This halophytic species typically occurs in the low to mid-elevations of the marsh, sometimes forming monotypic stands. This negative association supports the hypothesis that the low marsh areas dominated by *Salicornia* are not key habitat areas.

LPL3 stands as an outlier among the lagoon study sites. Three areas of Los Penasquitos Lagoon study site were examined, the first two contained skippers, the third did not. LPL3 is characterized by an assemblage of plant species similar to those occupied by wandering skipper as demonstrated with the NMDS analysis. *Frankenia* covers half the LPL3 plots with a large of area of *Distichlis spicata* (400 m^2) and open water within 15 m. The difference is that LPL3 is 2.3 km away from the tidal water at an elevation between 7.7 and 8.0 m above MSL. This area is never inundated by tidal waters and is best described as an alkali marsh rather than a salt marsh lagoon. In this study all observation of skippers occurred at elevations less than 7 m above MSL. This may be an important diagnostic indicator.

The one coastal bluff site was dominated by non-native plant species (58.5%). The dominant native species was *Distichlis spicata* (32%) with other native species contributing less than 2% cumulatively. *Cakile maritima* and to a lesser extent *Heliotropium curassavicum* were the dominant flowering plants where skippers were observed. Non-native weedy species such as *Carpobrotus edulis* (20.1%), *Myoporum laetum* (9.3%) and *Cortaderia jubata* (5.6%) were some of the key dominant species on the coastal bluff. Not surprisingly, *Frankenia* and *Salicornia* (species closely tied with coastal lagoons) were absent from the coastal bluff.

Nectar/Resting. This study did not determine the difference in plants being used for food (nectar) and those that were used for resting. Even so, the majority of skippers in the coastal lagoons were observed on *Frankenia* (85.2%). Compared to the availability within the study area, this plant species was used significantly more than by chance alone ($p = 0.0003$). *Frankenia* represents a key nectar source in the lagoon study sites. There seems to be a negative association with *Salicornia* and *Distichlis spicata*. This can be attributed to the lack of nectar available from these species. The only outlier was the BAT site for which skippers were observed 16% of the time using *D. spicata* compared to the 7% of available habitat.

On the coastal bluff, the skipper's ability to switch from *Frankenia* to *Cakile* and *Heliotropium* demonstrates the skipper's flexibility in use of nectar sources. Whereas other authors (Orsak, 1977; Busnardo, 1989; Brown, 1991) reported deerweed (*Acmispon*

Table 3. Species together contributing ≥ 90% of total abundance at each study site. All data are from transects, except BAT, LPL3 and TJ which are from plot data and shown for illustrative proposes. Transect and plot data were separated for cluster and NMDS analysis. Species shown in red italics are non-native species.

	AH	FAM1	FAM2	KFM	LPL1	LPL2	SEB	SEL	SWM1	SWM2	BAT*	LPL3*	TJ*
Frankenia salina	28.0	11.3	7.8	5.8	30.4	17.6		40.4	37.3	38.9	30.6	50.0	43.5
Sarcocornia pacifica	17.2	22.0	7.3	22.7	23.5	36.5		36.4	19.0	15.2	33.7		
Distichlis spicata	16.1	31.2	34.2			18.5	32.6	6.5	9.0	12.1		3.3	21.0
Distichlis littoralis		6.6		42.5					16.0	2.0			
Lollum multiflorum					39.1								
Batis maritima		8.9		15.2						3.5			
Jaumea carnosa		6.3	15.0					3.0					
Carpobrotus edulis						3.2	20.2						
Helminthathea echoides	21.5												
Arthrocnemum subterminale			4.7			2.8			4.2	9.1			27.4
Cuscuta salina		3.9	7.3			4.3		4.0					
Cakile maritima							15.9						
Cressa truxillenis	3.2								4.8	3.0	4.6		
Myoporum laetum							9.7						
Cortaderia jubata							5.8						
Limonium californicum				5.8									
Avena barbata			5.2							5.1			
Bromus hordeaceus													
Isocoma menziesii						4.8							
Bolboschoenus maritimus			4.7										
Juncus arcticus ssp. littoralis	4.3												
Juncus acutus			4.1										
Acacia sp.							3.5						
Arundo donax							3.5						
Atriplex semibaccata										2.5		10.7	
Spartina foliosa											14.4		
Artemisia palmeri											7.6		
Cyperus eragrotis												10.7	
Artemisia californica												6.6	
Salix gooddingii												6.6	
Malvella leprosa												4.1	

Table 3 (Cont.).

	AH	FAM1	FAM2	KFM	LPL1	LPL2	SEB	SEL	SWM1	SWM2	BAT*	LPL3*	TJ*
Ambrosia psilostachya						2.4							
Total	90.3	90.3	90.2	92.0	93.0	90.1	91.1	90.2	90.2	91.4	90.9	92.0	91.9

bold italic = non-native species

Table 4. Utilization of specific plant species for nectaring and/or resting by study site for all observed skipper in the study (n = 546). The percentage of plant species used (observed) by wandering skippers is compared to the total abundance of that plant species occurring in the study site was tested using a two-tailed, paired Student's t-test. Percentage data was arcsine transformed prior to running the t-test. Probability of occurring by chance alone is shown. Bold italics are significant at α = 0.05.

	Frankenia salina		*Distichlis spicata*		*Cakile maritima*		*Heliotropium curassavicum*		*Mesembryanthemum cystallinum*		*Carpobrotus edulis*		*Arthrocnemum subterminale*		*Salicornia pacifica*	
Study site	Observed	Available	Observed	Available	Observed	Available	Observed	Available	Observed	Available	Observed	Available	Observed	Available	Observed	Available
AH	91%	27%	0%	13%	0%	0%	0%	3%	0%	0%	0%	0%	9%	0%	0%	19%
BAT	84%	21%	16%	7%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	21%
FAM	100%	11%	0%	29%	0%	0%	0%	0%	0%	0%	0%	0%	0%	2%	0%	16%
KFM	100%	5%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	23%
LPL	94%	19%	4%	17%	0%	0%	1%	0%	0%	0%	0%	3%	0%	2%	2%	34%
SDL	94%	25%	6%	10%	0%	0%	0%	0%	0%	0%	0%	4%	0%	4%	0%	14%
SEL	96%	39%	4%	7%	0%	1%	0%	0%	0%	0%	0%	0%	0%	0%	0%	36%
SEB	0%	0%	3%	32%	73%	16%	21%	0%	0%	0%	3%	19%	0%	0%	0%	0%
SWM	93%	35%	0%	10%	0%	0%	0%	0%	7%	0%	0%	0%	0%	6%	0%	17%
TJ	100%	20%	0%	30%	0%	10%	0%	0%	0%	0%	0%	0%	0%	20%	0%	0%
Average	85.2%	20.3%	3.3%	15.5%	7.3%	2.7%	2.2%	0.3%	0.7%	0%	0.3%	2.6%	0.9%	3.5%	0.2%	17.9%
p=	**0.0003**		**0.01911**		0.45353		0.41604		0.34344		0.18901		0.28672		**0.00089**	

glaber), salty susan (*Jaumea carnosa*), chrysanthemum (*Chrysanthemum coronarium*) and goldenbush (*Isocoma*) as nectar sources, these were not documented in the present study. *Jaumea* was present in 7 of the 9 lagoon study sites, but skippers were never observed utilizing it. *Acmispon* and *Isocoma* are present along the transitional fringe of the lagoon, and despite extra effort to confirm the use of these plants, no skippers were observed nectaring on these. *Chrysanthemum* was not recorded from any of the study sites. *Frankenia*, *Cakile* and *Heliotropium* are the key nectar sources for wandering skippers, but the data suggest that the species may be opportunistic in regards to nectar source utilization.

A Conceptual Model. Based upon work done in this study, the author would like to offer a conceptual model for the habitat of wandering skippers. The primary habitat for wandering skippers consists of coastal lagoons and coastal bluffs (100%); elevations less than 5 m (98%); within 25 m of patches of *Distichlis spicata* over 1 m^2 (75%); and containing *Frankenia*, *Cakile* or *Heliotropium* (95%).

Conclusions

P. errans were found throughout San Diego in discrete areas of coastal lagoons and coastal bluffs. This study supports the observation that wandering skippers are not restricted to tidelands and estuarine habitat, and can occur along coastal bluffs. This study does not support the conclusion that the wandering skipper is dependent upon *Distichlis spicata* that is at least wetted by high tides (Nagano *et al.*, 1981), since observations of skippers using coastal bluffs were well above even the highest tides and storm surge. Conversely, skippers were not observed in what appeared to be suitable but unoccupied habitat (i.e., LPL3) well away from tidal influence. This study supports the hypothesis by Busnardo (1989) that the skippers favor lower and wetter, rather than drier areas of the salt marsh, but not areas of monotypic *Salicornia*.

This study lacked the ability to look at soil salinity or the potential physiological aspects of salt on the skippers, but the proximity to either tidal channels or salt spray from the ocean waves appear to influence the skipper's distribution. *Distichlis spicata* distance and the total amount present are important in the presence of the skipper and its abundance. While others have observed wandering skippers utilizing several species of plants for nectaring, this study concluded that 95% of the nectaring or resting occurred on just three species (*Frankenia*, *Cakile* or *Heliotropium*).

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Life history and Ecology of *Speyeria adiaste clemencei* (Comstock, 1925) (Lepidoptera: Nymphalidae)

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Abstract. In this paper we describe the life history and ecology of an endemic and declining California butterfly subspecies *Speyeria adiaste clemencei* (Comstock, 1925) from Chews Ridge, Monterey Co., CA. Despite its limited range, declining numbers, and one of the three *S. adiaste* subspecies already being extinct, a complete life history of this species has not been published. Our observations set the groundwork for future studies assisting in the conservation of this species. *S. a. clemencei* can be successfully reared on commercially available *Viola* spp., facilitating captive rearing for restoration. Larvae of *S. adiaste* can be distinguished morphologically from sympatric *S. callippe* and *S. coronis* larvae based upon coloration of the dorsal and dorsolateral scoli, head capsule coloration, and coloration of setae, facilitating identification in the field. We also document differences in adult behavior between the sexes and describe a shift in nectar source use during the observation period. We suggest that adequate access to nectar sources throughout the flight period, especially during drought years, as well as host plant density and distribution, are critical aspects for maintaining viable *S. a. clemencei* populations. These and other aspects of the ecology of *S. a. clemencei* warrant further study in order to better understand this imperiled species.

Keywords: larval ecology, immature stages, adult resource, endemic, *Viola purpurea*

Introduction

Butterflies are among the most well-known insects (New, 1997), and although early naturalists recognized the potential utility of immature stages in resolving taxonomic issues in butterflies (Müller, 1886; Edwards, 1877), the immature stages have been relatively understudied. Freitas and Brown (2004) have implicated various factors for this asymmetry, including a lack of larval specimens available in museums, low interest by lepidopterists, and the inherent difficulties in completing necessary fieldwork. Recent work on the morphological study and ecology of immature stages has proven valuable in resolving species boundaries, taxonomic issues, and cases of strong convergent evolution of adult phenotypes (Hill *et al.*, 2012; James & Nunnallee, 2011; Aiello, 2006; Freitas & Brown, 2004; Pech *et al.*, 2004; Brown & Freitas, 1994; Willmott & Freitas, 2006). Immature stages have also seen increasing attention in phylogenetic studies, but these often focus on a limited number of taxa (Penz *et al.*, 2013).

In addition to contributing to lepidopteran systematics, understanding larval ecology is crucial to the conservation of endangered species. This is particularly important for *Speyeria* butterflies, which have been in decline throughout the United States for the past 200 years (Hammond & McCorkle, 1983). For example, Bierzychudek *et al.* (2009) studied larval movement of endangered *Speyeria zerene hippolyta* to elucidate host-finding behavior. These caterpillars were unable to differentiate between their host plant and non-host plants at a distance of three centimeters, highlighting the need to focus on host plant density and spatial distribution in restoration efforts. *Speyeria zerene hippolyta* has recovered from near extinction, as a result of tree clearing, thatch removal, and mowing

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that successfully increased abundance of the *Viola adunca* host plant (Hammond, 1987, 1988, 1989; Hammond & McCorkle, 1991). Studying the effects of mowing regimes has also provided important insight into effective management practices in other butterfly species (Konvicka *et al.*, 2003; Johst *et al.*, 2006).

Studying larval ecology of *Speyeria* is of particular interest in California, where four of the thirteen federally-listed endangered or threatened butterflies are *Speyeria* taxa (USFWS, 2014). In addition to these four, an additional species, *S. adiaste*, has reportedly also been declining (Glassberg, 2001; Opler & Wright, 1999; Shapiro & Manolis, 2007; Scott, 1986; Kaufman & Brock, 2003). In contrast to the four federally-listed *Speyeria* taxa, the non-listed *S. adiaste* is the only *Speyeria* species endemic to California, and the only *Speyeria* species to have an entire subspecies population go extinct (with the possible exception of *Speyeria zerene myrtleae*, see Shapiro & Manolis, 2007). The unlisted status of *S. adiaste* has not gone unnoticed as there have been two petitions to have it listed, one in 1992 and the other in 2010 (USFWS, 2011). Despite its relatively narrow range and declining numbers, a complete life history for this species has not been published. According to Howe (1975), the life history for *S. a. atossa* was described by J. A. Comstock and C. M. Dammers and water color paintings were done by C. M. Dammers. Comstock and Dammers (1931) provide a description of *S. a. atossa*, but this is limited to the ultimate (6^{th}) instar, and the larva was not illustrated. Howe (1975) reports that the Dammers paintings were deposited in the Natural History Museum of Los Angeles County, but a search by museum staff was unsuccessful (R. P. Hulser, Chief Librarian pers. comm.). The lack of published information on this species, combined with its apparent decline and attempts to obtain listing status for *S. adiaste* motivated our current study of the life history and ecology of *Speyeria adiaste clemencei* at a well-known, long standing, and accessible population on Chews Ridge in Monterey Co, CA.

Materials and methods

Observations in this study were focused on the population at Chews Ridge, Monterey County, CA (N 36.31336°; W 121.57323°, 1500 m elevation). The habitat of *S. a. clemencei* at Chews Ridge is characterized as mixed oak-pine woodland. For identification of the *Viola* host plant and nectar sources found at Chews Ridge, we consulted Baldwin *et al.* (2012) and Matthews (2006). Observations on adult behavior were recorded during the course of adult censuses and a mark recapture study during the summers of 2012 and 2013. The behavior of each butterfly was recorded at the moment of capture. Egg duration was obtained by collecting eggs observed being laid by females in the field, which typically occurs in July and August. To obtain larvae for morphological study, wild-caught females were brought to the lab and placed in large brown paper grocery bags with dried *Viola* spp. similar to the methods of Mattoon *et al.* (1971). The dried host plant used to stimulate oviposition was a haphazard mixture of *V. papilionacea*, *V. pedunculata* and commercially available pansies (*Viola* spp.). We waited until late in the flight season to obtain worn females in an effort to minimize disturbance on the natural population. The females were fed one to two times per day on a 7% honey water solution soaked onto a sponge. They were allowed to feed until they were satiated. Females were preserved for genetic studies and the bag was closed when oviposition ceased. After two weeks the hatched larvae were removed from the bag, counted, and stored in wood blocks at 4°C for approximately four months. The wood blocks were one-inch by one-inch cubes of Douglas fir with a half-inch hole drilled completely through the center. The larval chamber was covered by fine mesh and stapled shut. The blocks were placed on dampened paper towel in an open plastic container. The blocks were misted semi-daily, and the paper towels changed frequently to prevent the accumulation of mold.

To break winter diapause of the first instars, each larva was placed into a one ounce opaque plastic Solo brand sauce cup lined with slightly damp paper towel that was re-wetted each day. Cups were placed under constant lighting approximately 14 inches below two 40W fluorescent lamps, and the larvae were stimulated twice daily using a small paintbrush. Larvae were fed commercially available pansies (*Viola* spp.) or *V. papilionacea* with the leaf petiole wrapped with damp paper towel. Larvae were fed *ad-libitum*. Larval head capsules and shed exuviate were collected at each molt and preserved in 70% ethanol. Notes on their behavior and morphology were taken daily. Photos of each stage were taken using a Nikon D80 camera, and 105 mm macro lens with extension tubes. Upon reaching the 5^{th} instar, larvae were transferred to larger two ounce plastic cups (Solo). Pupation occurred in these larger cups and pupae were subsequently attached to the lids of tall 10-ounce clear plastic drinking cups lined with paper towel for eclosion. Upon eclosion, adults were transferred to a 12-inch mesh cube to allow wings to harden before being frozen for preservation. Larvae of each instar were preserved in 70 % ethanol for morphological study. Images of head capsules were obtained using

a Leica S8 APO stereomicroscope with an attached Leica DFC295 camera. Leica Application Suite version 3.8 was used to measure head capsule images. Head capsule width was measured horizontally at its widest part, just above the dorsal-most stemmata in frontal view. Scanning electron micrographs of 1st instar morphology were taken with a Hitachi S-2600N scanning electron microscope (Hitachi High Technologies, Tokyo, Japan). The sample was first dried in an EMS 850 critical point dryer (Electron Microscopy Sciences, Hatfield, PA), using acetone as the intermediate solvent, and was then coated with gold in a Pelco SC-7 sputter coater (Ted Pella, Inc., Redding, CA), following manufacturer's protocols.

For the description of the larval stages, only one half of each segment was described. For each instar, at least two preserved specimens were used for morphological descriptions, supplemented with photos and descriptions from living larvae. When describing the larval scoli, we refer to three "rows" of scoli on each side of the midline. There is a dorsal row extending the length of the body, a dorsolateral row extending the length of the body, and a lateral row extending from A1 posteriorly. 1st instar chaetotaxy follows Hinton (1946), Kitching (1984), and Scott (1986).

To investigate the potential for field identification of larvae, we compared the larval color pattern in 2nd through 6th instars for the three sympatric *Speyeria* species found on Chews Ridge. The following 14 characters were used: proleg color, thoracic leg color, head capsule color, presence/absence of tan/brown patches on the head capsule, color of setae on scoli, color and thickness of dorsal line, body color, size and number of black body patches, presence of a lateral mottled gray line between dorsal and dorsolateral scoli, T1-A8 dorsal scoli color, T2-A8 lateral scoli color, A1-A8 dorsolateral scoli color, and the coloration of the A9 scoli and A10 scoli.

The observed behavior of adults was classified into five categories: chasing, search flight, direct flight, perching, and nectaring. These behaviors were distinct and could be unambiguously determined in the field. Chasing was a distinctly male behavior in which males would fly after other butterflies, insects, or birds. Individuals chasing each other would often circle one another rapidly flying upwards. Search flight was characterized as flying back and forth in an area at relatively low velocity, with no apparent overall direction. Search flight was clearly distinct from chasing and direct flight, and was used as a category for both males and females since it could be quickly categorized during mark recapture studies. However, it was clear that search flight behavior was distinct for males and females. Male search flight was characterized by flying back and forth in a small area, often moving in one direction then immediately circling back. The males would also occasionally land among vegetation for short periods before continuing their search flight. They seemed to be in search of females and/or nectar. Females in search flight flew much lower to the ground, and they often landed among vegetation and proceeded to walk around for short periods, before flying again and repeating the landing/walking sequence several times. This behavior was presumably an attempt to locate their host plant. Direct flight referred to a butterfly flying in a more-or-less straight line in one direction at a relatively high velocity without stopping or circling back in the opposite direction. Perching individuals were those seen sitting on the ground or vegetation, such as small shrubs or oak leaves, with wings opened or closed. Differences in nectar use and adult behavior were tested using Fisher's Exact Test (fisher.test) in R (version 3.0.2).

Results

Egg (Fig. 1A). Two eggs were measured. Egg 1: height (base to apex) = 0.96 mm, max width = 0.85 mm, height to width ratio = 1.1; egg 2: height = 0.94 mm, max width = 0.94 mm, height to width ratio = 1.0. Both eggs hatched in 14 days. Eggs are pale yellow-straw colored when laid, turning to brown after one to two days. The larval head capsule is visible within the egg the day before hatching. Egg is ovoid but relatively wide at base and tapered strongly at apex. Egg is about as tall as it is wide, and widest in basal third. Egg is sculptured with vertical ridges and resulting troughs; ridges occasionally bifurcate near base and are crossed at regular intervals by horizontal lines. Number of ova per female per day and total number of ova per female is unknown. One individual female laid 70 eggs in the lab during this study, but it is expected that females are able to lay up to a few hundred eggs in total, similar to that reported for other *Speyeria* species by James and Nunnallee (2011).

1st instar (Figs. 1B, 1C). Mean duration after ending diapause: 12 days (n = 18, range = 6-27 days, s.d. = 5.2). Mean head capsule width: 0.41 mm (n = 17, s.d. = 0.013). Head – Head capsule is sclerotized, dark brown to black with many brown setae. The setae protrude from a glossy dark brown to black sclerotized round base and have serrate projections. The stemmata appear black; the area enclosed by the ring of stemmata is a darker black on preserved specimens compared to the rest of the head capsule. Head capsule chaetotaxy is provided in Fig. 2. Body – Overall body pale brown freckled with many brown spots. The body is covered in setae and a 1st instar setal map is provided in Fig. 1B. Dorsal setae have bulbous tips (series D, XD, SD, and L), except for SD1 and L1 on A10, and setae have serrate projections. The body setae have conical bases. T1: Dorsally there is a dark brown to black sclerotized prothoracic shield located just posterior to the head capsule. The prothoracic shield has eight dark brown setae, four on each side of the midline (XD1-XD2, D1-D2). Laterally there are two dark brown sclerotized patches, with one (SD) superior to the other (L1-L2). Both of these are ovoid with two setae. There is a brown spiracle located posterior to the inferior-most lateral patch (L1-L2) that has a round opening at the apex of a conical protruding base, unlike the spiracles of the abdominal segments which are smaller and more flat. Ventrolaterally there is one dark brown sclerotized patch with two setae (SV1-SV2) protruding

from it. T2-T3: Dorsal to dorsolaterally there are three dark brown sclerotized patches (D1, D2, SD). The superior-most patch (D1) has two setae protruding from it whereas D2 and SD have only one seta. Laterally there is a dark brown sclerotized patch (L) with three setae. Ventrolaterally there is a dark brown sclerotized patch (SV1) with one seta. The thoracic legs (T1-T3) are heavily sclerotized, dark brown to black in color like the sclerotized body patches, with many setae protruding from the segmented areas of the legs. A1-A2: Dorsal to dorsolaterally there are three dark brown sclerotized patches (D1, D2, SD) that are similar in size and shape with the exception that the inferior-most of these three patches (SD) has one seta instead of two as seen on SD of T2-T3. Laterally there is an ovoid, dark brown sclerotized patch (L) with four setae. A small, ovoid, dark brown to black spiracle is located superior to L. The dark colored abdominal (A1-A8) spiracles stand out from the background body color. The spiracles on A3-A8 have the same morphology as on A1-A2. Ventrolaterally on A1-A2 there is a sclerotized patch (SV1) with one seta protruding from it. Ventrally on A1 there are two (one on each side of midline), very small, light brown sclerotized patches (P6), each with one small seta. Ventrally on A2 there are four (two on each side of midline) very small light brown sclerotized patches (P6 and V1) with one small seta. A3-A6: Dorsal to dorsolaterally there are three sclerotized patches (D1, D1, SD) that have the same morphology as the D1, D2, SD patches described in T2-T3. Laterally there is a sclerotized patch (L) with the same morphology as described for the lateral L patch in A1-A2. Ventrolaterally segments A3-A6 are devoid of sclerotized patches, but there are sclerotized patches on the prolegs. The prolegs are a pale brown, very similar in color to the body. Laterally each proleg has a dark brown to black sclerotized patch adorned with two setae (P2 and P4). A7-A8: Dorsal to dorsolaterally there are three patches (D1, D2, SD) with the same morphology as described for the D1-D2-SD patches in T2-T3. Laterally there is a sclerotized patch (L) with the same morphology as described for the lateral patch (L) in A1-A2. Ventrolaterally A7 and A8 both have one sclerotized patch (SV1) that is small, with one seta. Ventrally A7 has two (one on each side of midline) small, brown, ovoid sclerotized patches (P6) with one seta each, whereas A8 is devoid of patches ventrally. A9: Dorsal to dorsolaterally the morphology is the same as described for T2-T3. Laterally there is a dark brown sclerotized patch with one seta (L1). Ventrolaterally there is a brown sclerotized patch with one seta (SV1). A10: Dorsally there is a large dark brown to black sclerotized suranal plate with ten setae (five on each side of the midline) protruding from it (D1-D2, SD1, L1). There are no sclerotized patches in the lateral and ventrolateral regions. Ventrally there are ten small sclerotized patches some of which bear a single seta. These ten patches are arranged as two rows of four patches followed by a posterior-most row of two. The anal prolegs have large dark brown to black sclerotized patches laterally that bear many setae (P1-P4).

2nd instar (Fig. 1D). Mean duration: 6.7 days (n = 16, range = 4 to 9 days, s.d. = 1.4). Mean head capsule width = 0.60 mm (n = 17, s.d. = 0.026). Head – Very dark brown to black, numerous setae with two different general size classes. The smaller setae are more densely distributed near the mouthparts while the larger setae (~2x larger) are found superior to the stemmata on the head capsule. Setae are dark brown to black and have dark brown to black glossy cylindrical bases with bulbous ends. Stemmata appear black. Body – Overall the body is dark brown with cream colored mottling. Dorsally two cream colored stripes run the length of the larva. T1: The dorsal scolus projects overhead, particularly at rest. A second scolus lies dorsolaterally in the extreme posterior of T1 at border with T2. The dorsal scolus is entirely very dark brown to black. Superior to the dorsal scolus there is a black sclerotized prothoracic shield. Dorsolaterally there is a black sclerotized patch, roughly oval in shape with two setae. These setae are dark brown to black with conical bases and bulbous tips as seen on the head capsule and rest of the body. Laterally there is a black sclerotized patch that is more triangular in shape, and bears four setae. Just posterior to the lateral patch is the spiracle, with a black base and circular opening. Ventrolaterally there is a very dark brown to black oval-shaped sclerotized patch bearing three setae. T2-T3: Dorsal scolus colored as on T1. Dorsolaterally there are two black sclerotized patches, the triangular shaped anterior patch has four setae, and the posterior patch has two setae. Ventrolaterally there are three sclerotized patches; two larger patches with one superior to the other with four setae each, and a very small patch with one seta, located more anteriorly and superior to the thoracic legs. The thoracic legs contain sclerotized patches laterally. The sclerotized portions of the legs are black with non-sclerotized portions dark brown. A1-A2: Dorsal scolus as in previous segments. Dorsolaterally there is an entirely dark brown to black scolus roughly in line with the dorsolateral scoli of T2 and T3, but just slightly superior. Just inferior to this dorsolateral scolus, there is a spiracle that is much smaller than the spiracle found on T1. All of the abdominal segment spiracles are similar in size, shape, and color. They are ovoid in shape with a circular opening. Laterally there is a scolus with mixed coloration. The basal third is bright yellow, the middle third is a brownish-yellow and the distal third darkens to a black tip. Ventrolaterally there are two black sclerotized patches, one superior to the other, and both with three setae. The color of these patches is the same as all sclerotized body patches (dark brown to black), and the setae here are also dark brown to black. A3-A6: Same as A1-A2, except that there is only one dark brown to black sclerotized patch with two to three setae with 1-2 setae lateral to this patch. The prolegs are yellowish-brown in color and bear many small setae. A7-A8: Same as A3-A6, except that ventrolaterally there are two small sclerotized patches, both of which have two setae on A7, and one seta each on A8. A9: Dorsally the same as segments A7-A8. Dorsolaterally there is a sclerotized patch bearing one seta. Ventrolaterally there is one small sclerotized patch with one seta. A10: Dorsally the same as A9, but the scolus is shifted slightly inferior following the curvature of the segment. There is a black sclerotized suranal plate found in between the two dorsal scoli. Ventrolaterally there are several small sclerotized patches surrounding the anus and anal prolegs.

3rd instar (Fig. 1E). Mean duration: 6.6 days (n = 14, range = 4 to 12 days, s.d. = 1.8). Mean head capsule width = 0.91 mm (n = 17, s.d. = 0.034). The 3rd instar is a darker color with more black and less dark brown than the 2nd instar. The cream mottling on the body is much more apparent than on the 2nd instar. The arrangement and number of sclerotized patches and scoli are the same as in the 2nd instar. However there is an important change to coloration of some scoli. The orange coloration on the lateral scoli of A1-A8 is much brighter compared to the 2nd instar where the scoli gradually change from orange-brown to orange. The coloring of the rest of the scoli is the same; the dorsal scoli remain very dark brown to black throughout. The dorsal twin stripes are more apparent in the 3rd instar compared to the 2nd instar.

4th instar (Fig. 1F). Mean duration: 5.8 days (n = 12, range = 5 to 7 days, s.d. = 0.75). Mean head capsule width = 1.3 mm (n = 17, s.d. = 0.057). The arrangement and number of sclerotized patches and scoli are the same as in the 3rd instar. This instar has a more prominent orange coloration in the inferior-most lateral scoli of A1-A8 (due to increase in size). On the head capsule, superior to the stemmata and located to the left and right of the epicranial suture, are dark brown patches, sometimes with a stripe-like appearance. There is variation in the coloration of these patches with some individuals displaying tan to light brown colored patches on their head capsules. Similar small patches are found just superior to the base of both antennae. The mottling on the body is more apparent compared to 3rd instars, where the bright mottling near the base of the orange lateral scoli appears almost stripe-like when viewed from a distance with the naked eye.

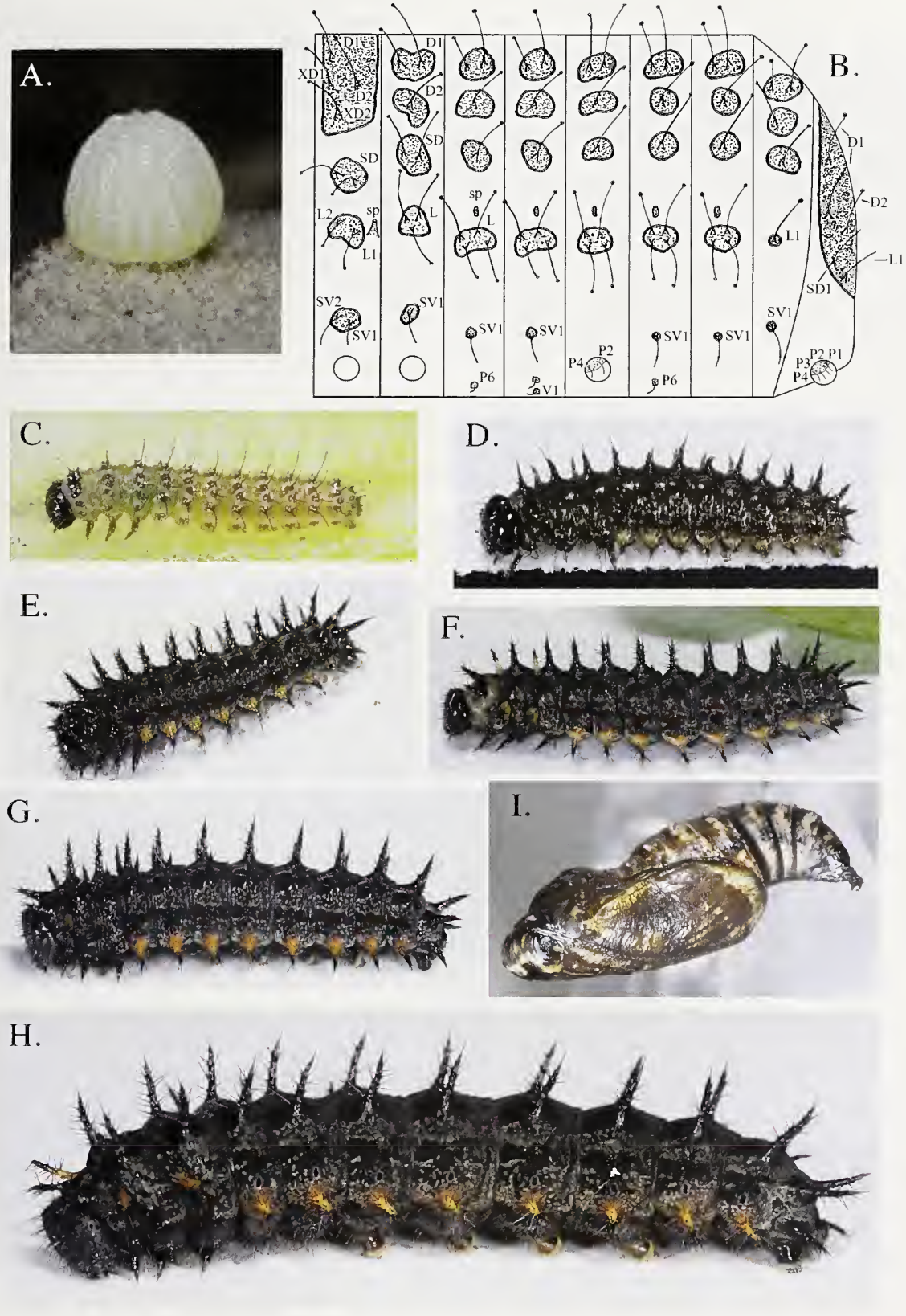

Figure 1. Immature stages of *S. adiaste clemencei*. (A) Egg (B) First instar setal map. Names of setae/patches belonging to the same row are indicated in the most anterior segment. (C) First instar (D) Second instar (E) Third instar (F) Fourth instar (mid-molt) (G) Fifth instar (H) Sixth instar (I) Pupa. Note: Images represent different individuals from the same brood.

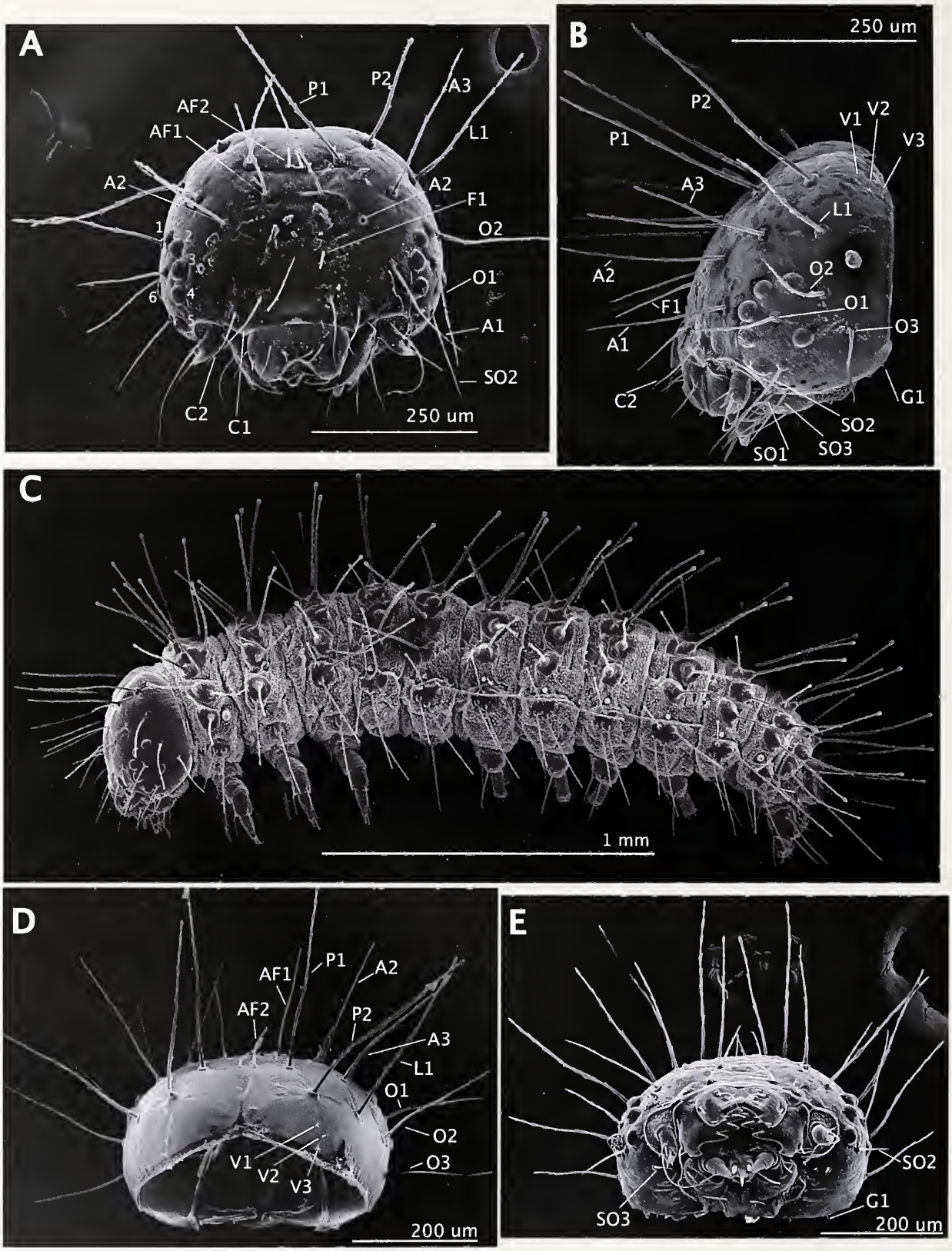

Figure 2. *S. a. clemencei* 1st instar head capsule and larval SEM images. (A) Head capsule - frontal view (Note: There is a broken seta between C1 and F1). (B) Head capsule - lateral view. (Note: round object superior to O2 seta is dirt) (C) 1st instar (D) Head capsule - dorsal view (E) Head capsule - ventral view.

5th instar (Fig. 1G). Duration = 5.5 days (n = 11, range = 4 to 7 days, s.d. = 0.82). Mean head capsule width = 2.0 mm (n = 17, s.d. = 0.083). The 5th instar is the same as previous instar except for an increase in body size.

6th instar (Fig. 1H). Mean duration: 10 days (n = 10, range = 8 to 15 days, s.d. = 2.0). Mean head capsule width = 2.9 mm (n = 5, s.d. = 0.091). Head – Head capsule is well-sclerotized, colored black and bears many black setae. The bulbed setae are either black with dark brown tips or entirely black. The setae on the head capsule are in two size classes, with the smaller setae being approximately one-fourth to one-half of the length of the larger setae. The larger setae tend to have brown tips. The majority of the larger setae are located dorsally. The setae have glossy black sclerotized round patches at the base. The brown stripe-like patches on the head capsule are more easily visible and are brighter than in previous instars. The stemmata appear black. Body – Body color is mottled charcoal gray and black. Twin cream-colored stripes run the length of the larvae dorsally. A relatively distinct mottled gray band lies between the dorsolateral and lateral scoli along the entire length of the larva. The prominence of the gray band is somewhat variable but strongest in the middle segments. T1: The dorsal scolus faces anteriorly and projects over the head, whereas the dorsal scoli of the other segments are upright and approximately perpendicular to the long axis of the body. Surrounding the dorsal scolus, there is a rectangular black patch, approximately two-thirds of which lies anterior of the scolus and one-third lies posterior of the scolus. The basal 33-40% of the dorsal scolus has a dark brown color that turns to black in the upper 60-67%. The dorsal scolus has approximately twenty setae along its shaft. The setae have bulbous ends and are of equal length. The scolus terminates with a ring of three to four setae, which are shorter than the rest of the setae on the scolus, and a single terminal mid-length seta. The setae are black in color and at the base of each seta is a glossy-black patch where it extends out of the scolus. Superior to the dorsal scolus is the prothoracic shield, which is less prominent in this instar than in younger instars. Laterally there are two dark brown to black sclerotized patches. The superior-most lateral patch is circular in shape and bears six black setae. Directly inferior to this patch, is the second somewhat larger and more triangular shaped patch with approximately ten black setae protruding from it. Posterior to the triangular sclerotized patch is the first spiracle. This spiracle is slightly larger than the rest of the spiracles on A1-A8. The ovoid T1 spiracle is black which stands out against the background body color, which is not as dark. Ventrolaterally there is a small triangular sclerotized patch with five to six black setae protruding from it. Ventrally and anterior to the two thoracic legs is the eversible neck gland, which emits a foul odor when the caterpillar is disturbed. The thoracic legs are dark brown to black and well-sclerotized. T2-T3: The dorsal scolus is the same color as the T1 dorsal scolus and points vertically, perpendicular to the long axis of the body. Dorsolaterally there is a small black sclerotized patch with approximately ten setae protruding from it. Laterally there is a black sclerotized patch with approximately ten setae protruding from it. Anterior and slightly inferior to the dorsolateral patch, there is a scolus located at the boundary between segments T1 and T2, and an identical scolus lies between T2 and T3 in the same plane. The basal 60% of these scoli are orange with the apical 40% black. Ventrolaterally there are three ovoid black sclerotized patches located just above the thoracic legs. One of these is smaller and more anterior and all three have five to ten setae protruding from them. The thoracic legs are the same as on T1. A1-A2: The dorsal scolus on each of these segments is entirely black. Unlike T2-T3, there is not a dorsolateral sclerotized patch. Dorsolaterally there is a scolus with the basal 25-33% colored dark brown, middle 33-50% orange-brown, with the remaining distal portion black. Laterally there is a second scolus just inferior to the spiracle, with the basal 80% bright orange and apical 20% black. There is variation in the coloration of that lateral scolus; some individuals go from the bright orange to a more yellowish or tan color above 20% basally as opposed to the more uniform basal 80% bright orange, apically the black is constant. The spiracle is ovoid and black. Ventrolaterally there are two roughly ovoid black sclerotized patches, one superior to the other, and each bearing approximately eight setae. Ventrolaterally and ventrally there are numerous scattered setae. A3-A6: Same as A1-A2, except that ventrolaterally there is only one black sclerotized patch instead of two. The prolegs are pale orange to tan, with a dark brown to black elongate sclerotized patch laterally bearing numerous setae. A7: Same as A1-A2, except the two black sclerotized patches are relatively smaller. A8: Same as A3-A6, except that the ventrolateral black sclerotized patch is smaller and located more inferiorly. A9: Dorsally the same as A1-A8. Laterally there is a black sclerotized patch with four setae. Ventrolaterally there is a black sclerotized patch with three setae. A10: The only scolus in this segment is located laterally and is completely dark, resembling the dorsal scoli of segments A1-A9. The posterior portion of A10 contains a black sclerotized suranal plate (above the anus). Posterior to the anus is the anal proleg that is colored the same as the A3-A6 prolegs but has a relatively larger basal black sclerotized patch laterally which bears numerous setae.

Pupa (Fig. 1I). Mean duration: 15 days (n = 8, range = 14 to 17 days, s.d. = 1.0). Upon initial pupation, the pupa is orange without any dark brown and cream-colored patches, and then darkens within 24 hours. The mature pupa is nearly entirely dark brown with a small amount of cream colored mottling. The cremaster is black. Dorsally the head is mainly cream colored with dark brown spots where the eyes are located. Antennae are found ventrally between the wings, and they have alternating patches of dark brown and cream-colored patches. Ribbed portions of the antennae are visible. The two halves of the proboscis are found in between the antennae and are nearly entirely dark brown. Where the proboscis meets the head ventrally there is a dark brown bean shaped patch on the head. Posterior to the head, there are two raised cream-colored diamond-shaped tubercles dorsally. The thorax dorsum is raised and keel-shaped with an additional raised line down the midline. Dorsally the abdomen has alternating dark brown and cream-colored roughly triangular patches. The abdominal segments have two dorsal rows of tubercles on each side of the midline. These tubercles form the points of the dark brown triangular patches anteriorly. The dorsal anterior brown triangular patches vary in size and shape between individuals; the brown anterior triangular patches are sometimes joined to smaller brown triangular patches posteriorly with the cream-colored triangular patches in between these giving them a more pentagonal rather than triangular shape. Dorsally, along the midline within the cream colored central triangular patch of each abdominal segment, there are two dark brown circles in between the two dorsal tubercles. Smaller dark brown tubercles are found both superior to and inferior to the spiracles on segments A1-A8. Lateral and ventrolateral portions of segments A4-A6 have a dark brown band where the segments articulate. The wing case is dark brown with small amounts of cream-colored patches around the border of the wings, with some patches in the middle as well.

Differentiation of sympatric *Speyeria* larvae

Overall our observations indicate that *S. adiaste* can be distinguished from both *S. callippe* and *S. coronis* in the 4th, 5th and 6th instar by the presence of mottled brown patches on the head capsule. We did not observe discernible differences in larval color

pattern among *S. a. clemencei*, *S. c. comstocki* and *S. c. coronis* in the 2nd or 3rd instars. In the 4th instar the body of *S. a. clemencei* appears to be darker and less strongly marked than *S. c. comstocki* and *S. c. coronis*, which have brighter colored body mottling overall, as well as whitish-orange patches near the dorsal scoli (*S. c. coronis* has more orange colored patches near the dorsal scoli compared to *S. c. comstocki*) and a more prominent gray to whitish mottled lateral band between the dorsolateral and lateral scoli. In the 5th instar *S. adiaste* and *S. callippe* resemble one another in body coloration too much to differentiate them, with both being fairly darkly colored. *S. c. coronis* 5th instars look very similar to their 4th instars, which makes it easy to distinguish it from the other two species in this stage. In the 4th and 5th instar *S. a. clemencei* develop mottled brown patches on the lateral and dorsolateral area of the head capsule that are absent in *S. c. comstocki* and *S. c. coronis*.

In the sixth instar larvae of all three species can be well-distinguished morphologically. Of the 14 characters examined, the extent and brightness of orange coloration at the base of the dorsal and dorsolateral scoli, the presence/absence of brown/tan patches on the vertex of the head capsule and the color of the setae on the scoli serve to separate the species (see Table 1 and Fig. 3). *S. a. clemencei* consistently had the darkest colored dorsal and dorsolateral scoli (Fig. 3) and was the only one of the three species to have the brown/tan colored patches on the vertex of the head capsule. In contrast, *S. c. coronis* was brighter overall in coloration and had much more orange coloring in both the dorsal and dorsolateral scoli, and was different in that it had brown colored setae on the scoli, compared to black setae for the other two species. *S. c. comstocki* was intermediate in coloration and similar to *S. a. clemencei* in that it appeared dark overall, however it consistently had more orange coloration in its scoli (see Table 1 and Fig. 3).

Adult biology

S. adiaste clemencei individuals were observed nectaring on five different nectar sources on Chews Ridge during the 2012 and 2013 flight seasons (Fig. 4). Combining both years, 68% (82 of 120) of the individuals nectared on *Cirsium occidentale* (Asteraceae), and 28% (33 of 120) nectared on *Monardella villosa* (Lamiaceae). The remaining nectaring records included *Asclepias eriocarpa* (Asclepiadaceae; 3 of 120; 3%); *Verbena lasiostachys*, (Verbenaceae; 1 of 120, 1%); and *Wyethia helenioides* (Asteraceae; 1 of 120; 1%). We observed a marked shift in the frequency of the two main nectar sources used between 2012 and 2013 (Fisher's Exact Test, $p = 0.0004$). In 2012, *C. occidentale* made up 78% (72 of 92) of the observations, compared with 37% (10 of 28) in 2013. *M. villosa* comprised 20% (18 of 92) of the nectar records in 2012, compared with 55% (15 of 28) in 2013. There were no observations of *S. adiaste* adults nectaring on *V. lasiostachys* or *W. helenioides* in 2012 (Fig. 4).

Table 1. Variation in the key diagnostic characters for sixth instars of three sympatric *Speyeria* species at Chews Ridge. N = # of larvae used for the description. Other characters studied were too variable to allow reliable determination.

Character	*S. adiaste clemencei*	*S. callippe comstocki*	*S. coronis coronis*
T1-T3 Dorsal Scoli	Basal 0-40% pale tan to dark brown; Apical 60-100% black. N = 12	Basal 50% gray-brown to brownish-orange; Apical 50% black. N = 8	Basal 50-65% pale gray to orange; Apical 35-50% black N = 1
A1-A10 Dorsal Scoli	Basal 0-40% pale tan to dark brown; Apical 60-100% black. N = 12	Basal 50-80% gray-brown to brownish-orange; Apical 20-50% black. N = 8	Basal 80% pale-orange to orange; Apical 20% black. N = 1
A1-A8 Dorsolateral Scoli	Basal 20-25% brown to dark brown; Apical 75-80% pale yellowish-brown to orange-brown grading into black. N= 12	Basal 80% yellowish-orange to orange; Apical 20% black. N = 8	Basal 80% orange; Apical 20% black. N = 1
Brown/Tan Patches on Vertex of Head Capsule	Present, head capsule is black with narrow patches of tan/brown present on vertex and dorsolaterally N = 12	Absent, head capsule is all black. N = 5	Absent, head capsule is all black. N = 1
Setae on Body Scoli	Black N= 12	Black N= 5	Brown to dark brown. N= 1

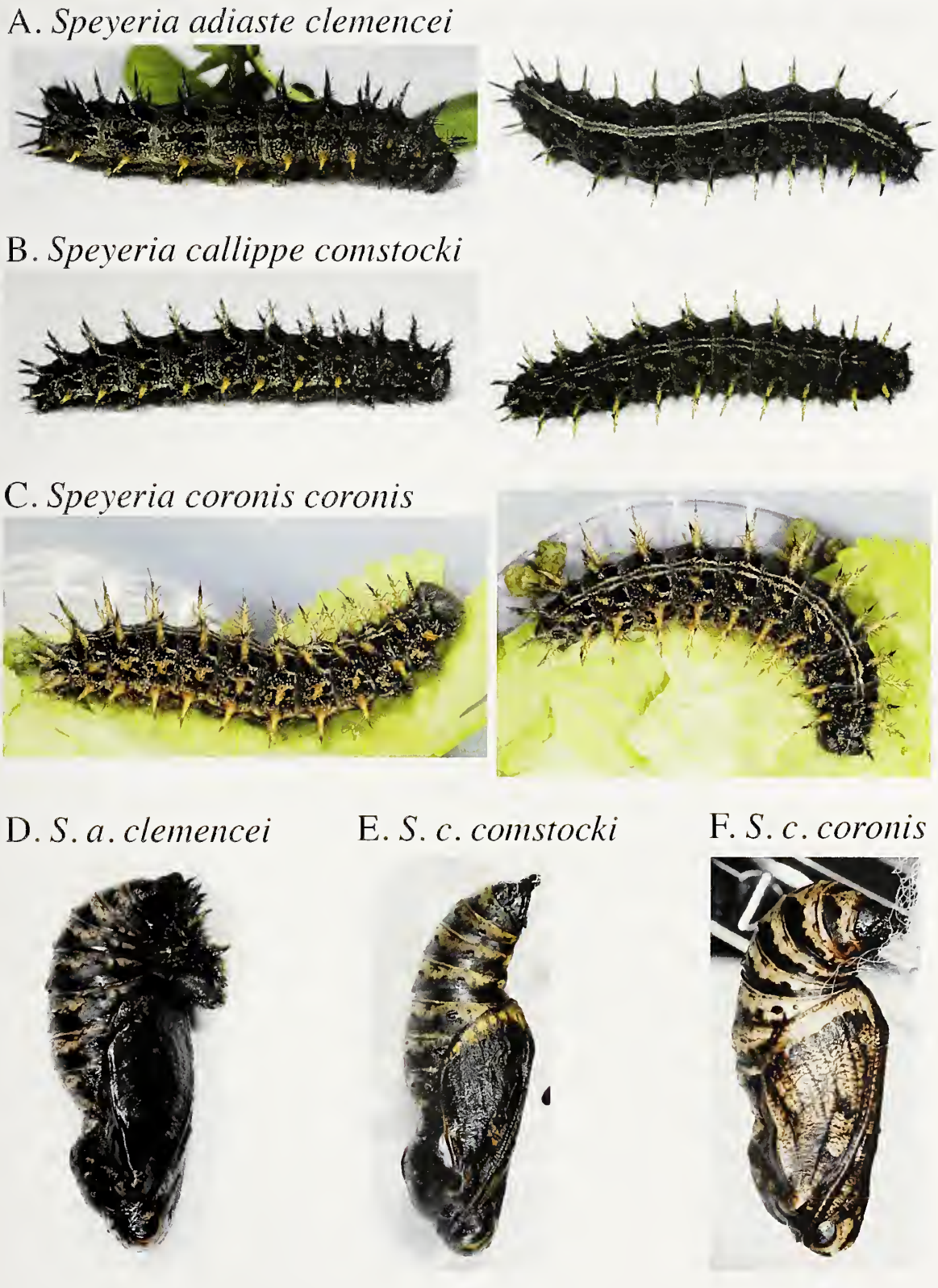

Figure 3. Ultimate instar (6th) and pupa of the three *Speyeria* species found at Chews Ridge, Monterey Co., CA. On the left side (A-C) is the lateral view and right side is the dorsal view. (A) *S. a. clemencei.* (B) *S. c. comstocki.* (C) *S. c. coronis.* In the 6th instar, *S. a. clemencei* can be distinguished by its darkly colored dorsal and dorsolateral scoli compared with the other two species. (D-F) are lateral views of *S. a. clemencei, S. c. comstocki* and *S. c. coronis* respectively. *S. adiaste* and *S. callippe* are quite similar in pupal morphology.

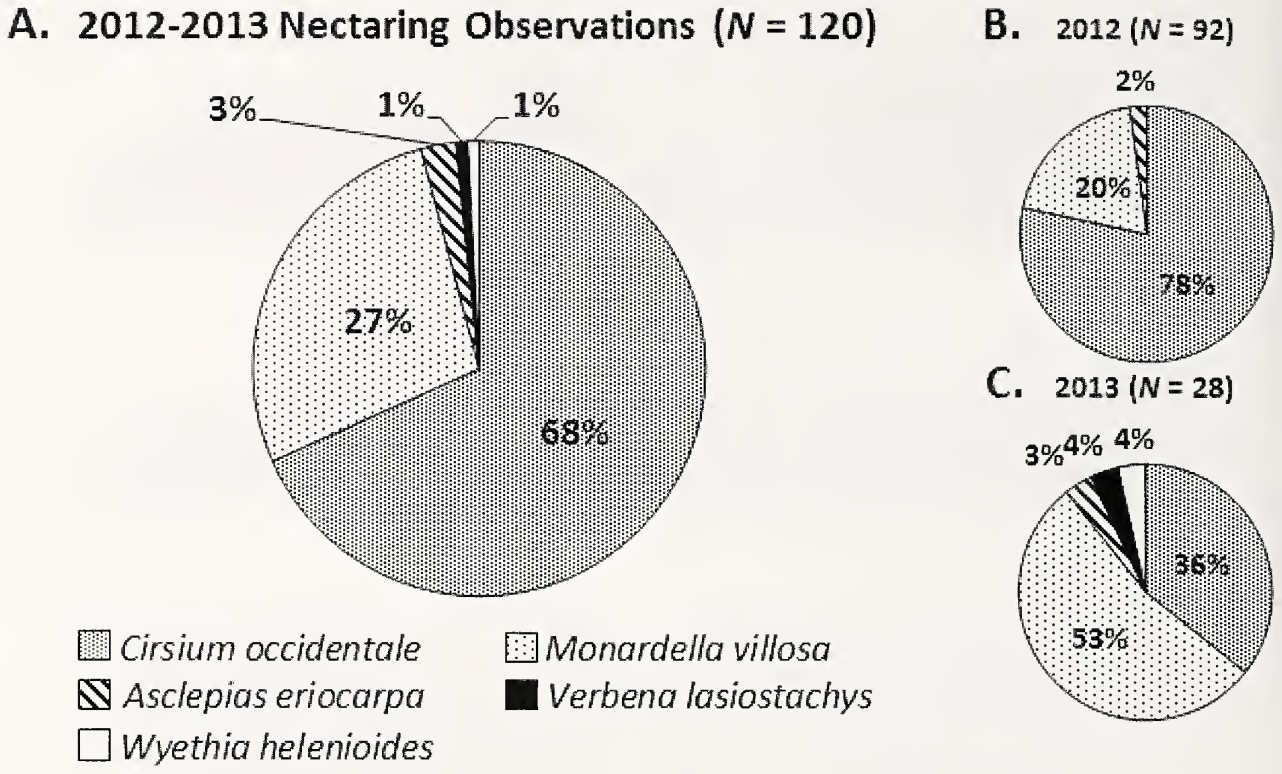

Figure 4. Adult nectar observations on Chews Ridge. (A) Combined data from 2012-2013 (N = number of observations). (B) Nectaring observations from 2012 only. (C) Nectaring observations from 2013 only. Note the relative difference in *Cirsium* and *Monardella* between years.

We observed a significant difference in behavior between males and females (Fisher's Exact Test, $p < 0.0005$; Fig. 5). The most common male behaviors were search flight (39%, 196 of 506), direct flight (28%, 143 of 506), and nectaring (20%, 101 of 506). Females were also often observed in search flight (34%, 42 of 125), however only 4% (5 of 125) in direct flight, and 13% (17 of 125) nectaring. Females were observed perching (49%, 61 of 125) more than males (7%, 37 of 506). One male behavior not observed in females was chasing other butterflies and insects, occasionally even hummingbirds (6%, 29 of 506).

Ovipositing females typically landed near areas with the host plant *V. p. quercetorum*. Upon landing, a female dragged her abdomen along the ground, simultaneously shivering and flapping her wings as she walked. Females seemed to "false oviposit" quite often, as suggested by an extreme curling of their abdomen as if probing for the host plant or appropriate substrate. Females spent most of their time near senescing *Viola*. They walked under broken branches and twigs, into ground squirrel holes, under non-host plants, and under leaf debris to oviposit one egg at a time. The females seemed to prefer shadier areas with dappled sun under and near deciduous and non-deciduous oaks (*Quercus* spp.). Females appeared to begin ovipositing right away during their relatively short time on the wing, indicating an absence of reproductive diapause, in contrast to sympatric *S. coronis coronis* (R. Hill & C. Tenney, pers. observ.; Shapiro & Manolis, 2007.) and *S. coronis snyderi* in northern California (Sims, 1984).

Discussion

The goal of this study was to document larval morphology, ecology, and other aspects of the biology of *S. adiaste*, to lay the groundwork for further studies and to help inform conservation decisions regarding this species. Given that *S. adiaste* is sympatric with two other *Speyeria* species, simply finding and correctly identifying a *S. adiaste* larva in the field is an important first step. In addition, a better understanding of the life history traits and natural history of *S. adiaste* can identify the most critical aspects of its biology to guide conservation efforts. It is our hope that this study encourages study of immature stage biology and demonstrates the importance of natural history observations for increasing the understanding of species of conservation concern.

Larval morphology and ecology

One challenge to conducting fieldwork on larval ecology is species identification. Previous authors have stated that *S. callippe* and *S. adiaste* larvae are difficult to distinguish (Scott, 1986; Dunford, 2009; Allen *et al.*, 2005). Our results indicate that *S. adiaste* and *S. callippe* larvae are very similar, however, based upon examining 14 characters, we found that *S. a. clemencei* can be separated from the other two species in the 4th and 5th by head capsule color, and all three species can be distinguished in the 6th instar by coloration of the scoli, coloration of setae on the scoli, and the presence/absence of brown/tan patches on the head capsule (Table 1, Fig. 3). The lack of clear color pattern differences in 2nd to 3rd instars between species hampers field identification and indicates rearing to at least the 4th instar is necessary for correct determination. However, additional traits other than color pattern could be studied for differences, but their utility would be limited for field identification as a microscope will likely be needed. The characters useful for separating 4th, 5th, 6th instar *S. adiaste*, *S. callippe* and *S. coronis* observed here are consistent with Comstock and Dammers (1931, p. 44) observations on morphological differences between 6th instar larvae of *S. a. atossa*, *S. callippe macaria*, and *S. coronis semiramis*. However, the "more pronounced lateral mottled gray line" that Comstock and Dammers (1931) used to distinguish *S. a. atossa* from *S. c. macaria* and *S. c. semiramis* appears variable within *S. a. clemencei*, making it a less useful diagnostic character to separate 6th instars on Chews Ridge. In the 6th instar the majority of the *S. a. clemencei* individuals had a prominent gray mottled band between the dorsal and dorsolateral scoli, but a few were weak, and some very weak. There was less variation within *S. callippe comstocki* for this character, with the general trend being that the gray mottled band was much less conspicuous in most larvae, however, one individual did have a prominent gray band. In the 6th instar *S. coronis coronis* appears distinct from the other two species on Chews Ridge in having a grayish band with orange/tan mottling.

Despite the utility of the coloration of the dorsal and dorsolateral scoli for identification, there was variation in these characters within broods and among broods. Given our relatively small sample size, additional observations may be needed to confirm the overall pattern of differences in these characters among the sympatric species. Two broods of *S. a. clemencei* larvae were used for the morphological description, with two broods for *S. c. comstocki* and one brood for *S. c. coronis*. Furthermore, only a single specimen of *S. c. coronis* was used for the morphological comparisons. A larger sample size from multiple broods for *S. c. coronis* may be needed to understand phenotypic variation within this species; however, due to the strong morphological distinction between *S. a. clemencei* and the *S. c. coronis* larva, it does not seem likely that the *S. c. coronis* larval phenotype will broadly overlap with further observations. In contrast, *S. a. clemencei* and *S. c. comstocki* larvae were more similar, and variation within and among the *S. c. comstocki* brood and *S. a. clemencei* broods may make it challenging to identify these two species in the field (see Table 1 for details on variation). Although we were confident in using the dorsal and dorsolateral scoli as a means of separating 6th instar *S. a. clemencei* and *S. c. comstocki*, further observations with multiple broods would help provide a more complete understanding of variation in the immature stages of

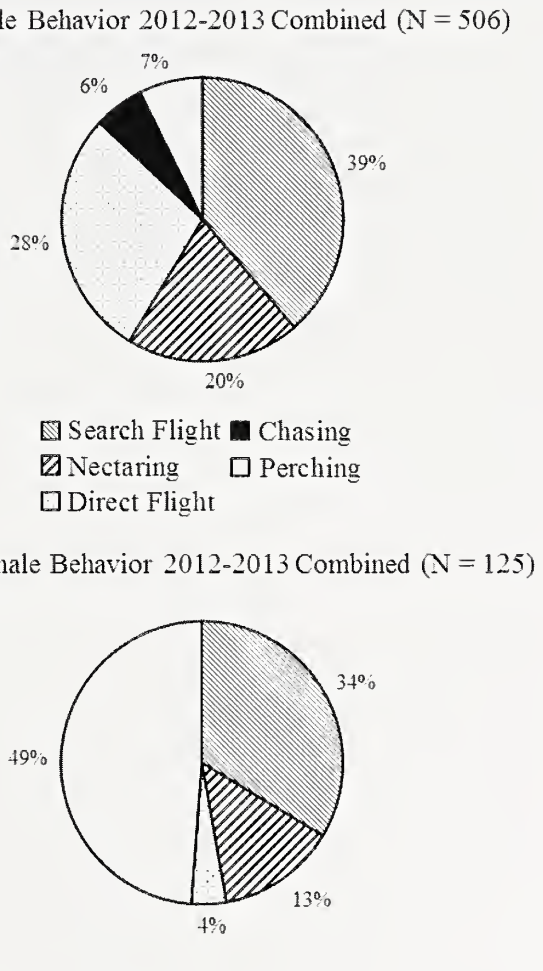

Figure 5. Adult behavior on Chews Ridge. (A) Observed male behaviors combined from 2012-2013. (B) Observed behavior for females during 2012-2013. N = total observations, not necessarily unique individuals.

these two species. The fact that Comstock and Dammers (1931) identified similar characters for differentiating between *S. a. atossa* and *S. callippe macaria* (and *S. coronis semiramis*) suggests that the traits are broadly useful between species (see also Sims *et al.*, 1979).

Although some instars of *S. adiaste* can be separated from sympatric *Speyeria* on Chews Ridge, additional field studies of *S. adiaste* larvae are challenging due to difficulties in locating the larvae. *Speyeria* larvae are known to be difficult to find in the field, possibly due to their nocturnal feeding habits (Shapiro & Manolis, 2007; Allen *et al.*, 2005; Comstock, 1927) and our results searching for *S. adiaste* are consistent with this. On May 1st, 2012, we searched areas with dense *Viola purpurea quercetorum* host plant on Chews Ridge from 19:30-22:30 (three observers = nine person-hours total search time). Based on the date of appearance of the first adults in 2012, and using the average duration of each instar based on lab rearing (see Results), most larvae in the population should have been in the 4th instar by this date, indicating they should have been fairly conspicuous during the search. However, we only found a single 4th instar larva with this effort. Furthermore, if larvae were easy to find, they should have been encountered in April and May of 2013 during an intensive survey of *V. purpurea* abundance on Chews Ridge. The survey was conducted during daylight hours and we did not find a single larva in 270 person-hours spent counting *V. purpurea*, despite commonly finding evidence of herbivore damage on the leaves. It is possible that larvae were overlooked during the survey in early April when they would have been very small 1st/2nd instars, which would have been very difficult to find due to their size and coloration. However, by mid-May, larvae should have been in the 6th instar, and therefore, much more conspicuous during the survey effort. Overall, our observations suggest finding larvae in the field is challenging, making field studies of larval ecology particularly difficult.

The difficulty finding larvae in the field places a premium on captive rearing of this species in order to better understand its larval ecology. Our studies indicate that *S. adiaste* can successfully be reared in the laboratory by using commercially available *Viola* spp. (*V. tricolor* and hybrids). For a rare butterfly species such as *S. adiaste*, successful rearing in the lab allows for the possible implementation of a captive rearing program for restoration purposes. Currently there are many organizations that are involved in rearing rare and endangered butterfly species for reintroduction into the wild (James & Nunnallee, 2011). Captive rearing has been shown to be a robust method to maintain severely at-risk populations in the short-term, though it does not appear to be a viable long-term solution (Crone *et al.*, 2007). We loosely followed rearing methods described by Mattoon *et al.* (1971) and Wells *et al.* (2011). However, there was likely room for improvement in our rearing methods that could have led to increased oviposition. During our rearing, we obtained 70 ova from a wild-caught *S. adiaste* female captured late in the season. We used only a single full-sized grocery bag during oviposition, in contrast to Wells *et al.* (2011) method of replacing the bag each day; this could have limited the number of eggs laid. Furthermore, although females were given 7% honey-water solution and fed *ad libitum* in lab, no amino acids were added. The addition of amino acids could promote increased oviposition by supplementing sub-optimal larval food resource conditions the females might have encountered in the wild (Mevi-Schutz & Erhardt, 2003; Wagner *et al.*, 1997).

The life history strategy and larval ecology of *S. adiaste* have important implications for the persistence of its populations. *Speyeria* butterflies appear adapted to areas with dense, well-connected patches of their *Viola* host plant, where larvae can quickly locate a host and roam from plant to plant, and larval mortality is mitigated by high fecundity. *Speyeria* females oviposit single eggs on various substrates near dry or senescing violet host plants, but very rarely oviposit on *Viola* itself (Wagner *et al.*, 1997; Kopper *et al.*, 2000). The overwintering larvae do not eat their host until the following spring, and must locate suitable host after months of diapause, making this a critical step for larval survival. Increased larval mortality is likely if patches of host plant are small, patchily distributed or do not have adequate density. Laying large numbers of eggs is likely a strategy to overcome the high mortality rate of overwintering first instars (Wagner *et al.*, 1997; Kopper *et al.*, 2000). *S. idalia* and *S. diana* may represent the upper limit of fecundity within *Speyeria*, laying from 1000 eggs to occasionally over 2000 eggs in their lifetime, some of the highest amounts ever recorded from butterflies under laboratory settings (Wagner et al., 1997; Wells *et al.*, 2011). Our observations suggest that this level of fecundity probably does not occur in *S. adiaste*, which likely lays several hundred eggs, rather than thousands. This is more consistent with other *Speyeria* species that have been shown to oviposit between 100-1000 eggs (James & Nunnallee, 2011). The lower fecundity of *S. adiaste clemencei* suggests mortality of overwintering larvae is a critical aspect of population persistence. Overall, the effectiveness of this life history strategy appears to be limited for many current *Speyeria* populations, since many members of this genus are reportedly suffering due to habitat degradation and perturbations of their *Viola* host plants (Hammond & McCorkle, 1983).

Habitat loss and changes in *Viola* density and distribution likely present difficult challenges for *Speyeria* larvae, which are limited in their ability to detect and find host plants (Bierzychudek *et al.*, 2009). In a field-based study of host-finding capabilities of endangered *Speyeria zerene hippolyta* larvae, Bierzychudek *et al.* (2009) found that larvae were unable to distinguish their host plant from non-host at distances of three centimeters. Weiss and Murphy (1988) provide further evidence of the difficulties faced by early instars in their attempts to find food. Their model, using simulations of a rough-textured grassland environment, indicated that one to two millimeter sized caterpillars, similar in size to first instar *Speyeria* larvae, would need to walk 42 meters in order to cover one meter of linear distance. These results may be particularly important for *S. a. clemencei* on Chews Ridge, as their host *Viola purpurea* is abundant, but very patchily distributed (K. Zaman, C. Rush, C. Tenney, R. I. Hill, unpublished). This patchy distribution may make it difficult for first instar *S. adiaste* to locate their host plant if they have similar host finding abilities to those of *S. z. hippolyta*. As suggested by the studies of Bierzychudek *et al.* (2009) and Wagner *et al.* (1997), in order to counteract the lack of efficient host finding capabilities of *Speyeria* larvae and the oviposition behavior of females, dense patches of their *Viola* host plant are necessary for larvae to successfully find food and prevent starvation. In order to better understand the possible restoration requirements for *S. adiaste*, we recommend study of larval movement patterns and host finding abilities and their relation to the abundance and distribution of their *Viola* host plant.

Habitat and host plant

In order to obtain a clearer view of *S. adiaste* across its range, it is useful to compare our observations for *S. a. clemencei* with available information on the habitat and host plant requirements for the remaining subspecies. Across the range of *S. adiaste*, there appears to be a trend toward higher elevation and more open habitats southward (Scott, 1986). Scott (1986) suggests that the northern *S. a. adiaste* inhabits redwood forest openings, which corresponds with Comstock's (1927) description of *S. a. adiaste* as a "forest lover, delighting in the flowered glades adjacent to the redwood groves." Howe (1975) corroborates that *S. a. adiaste* is "more closely associated with forests than either *clemencei* or *atossa*." *S. a. clemencei* at Chews Ridge inhabits mixed oak-pine woodland. Hovanitz (1970) describes the habitat of *S. a. clemencei* as "openings in oak woodland," and indicates that both *S. a. adiaste* and *S. a. atossa* share this same type of habitat. *S. a. atossa* was apparently more similar to *S. a. clemencei* in that it inhabited more open habitats (Howe, 1971; Comstock, 1927).

We observed *S. a. clemencei* on Chews Ridge using *Viola purpurea quercetorum* (Baldwin *et al.*, 2012) as its host plant. Chews Ridge females were observed ovipositing near *V. p. quercetorum* growing in shaded areas under oaks. In comparison, *Viola pinetorum* has been listed as a possible host plant for *S. a. atossa* (apparently misspelled "*pinctorum*" Comstock 1927, p. 89), due to its abundance in fields with emerging *S. a. atossa* adults (Comstock, 1927). The host plant for *S. adiaste adiaste* is unclear; Scott (1986) and Robinson *et al.* (2002) suggest *Viola ocellata*, one of several *Viola* species found within the range of *S. a. adiaste*.

Adult ecology

Behavioral differences between sexes is common among adult Lepidoptera, which can lead to differential use of habitat and adult resources (Scott, 1986). There was a clear difference in the observed behavior of male and female *S. a. clemencei*. Males flew faster and higher above the ground, and were thus more visible to observers. Females typically flew low to the ground and were constantly landing and searching. Males were also observed relatively more often in flight-related activities (Fig. 5), increasing the probability of encounters with the observers. Male observations involving flight (search flight, direct flight, and chasing) comprised 73% (368 of 506) of male observations, compared with 38% (47 of 125) flight-related observations (search and direct flight) for females (Fig. 5). Males were most commonly observed in search and direct flight, presumably to locate females (Fig. 5). Females were most commonly observed in behaviors related to finding host plants and oviposition, as perching and search flight made up 49% and 34% of our female behavioral observations respectively.

It is important to note that the calculated percentages for each observed behavior may not reflect the relative amount of time that males and females were engaged in that particular behavior outside of our observation periods. The conspicuousness of flight behaviors indicates that this alone could cause these behaviors to be highest in frequency. Still, even among conspicuous flight behaviors there were relative differences between males and females, such as little direct flight and no chasing in females, that is indicative of differences between the sexes. Furthermore, if our observations were simply the result of detecting the most conspicuous behavior, such as those involving flight, then perching should not have been as commonly observed in females. If we assume the frequency we observed for each behavior was constant over the entire time that the butterflies were active during the day, the increased

time in flight for males may be associated with increased predation risk, and higher-energy expenditure. The relatively less conspicuous behavior of females likely keeps them out of sight of predators, and may conserve energy for egg production.

In addition to host plant ecology, adult nectar sources may play an important role in population persistence of *S. adiaste*. Published nectar plant records of *S. adiaste* include various thistles (*Cirsium*, *Carduus*, and *Silybum*), tansy ragwort (*Senecio jacobea*), and *Brodiaea* (Shapiro & Manolis, 2007). For *S. a. clemencei*, Howe (1975) listed *Aesculus californica* and *Eriodictyon californicum* as nectar sources. These plants are not present at the Chews Ridge study site, but rather are found at lower elevations nearby (R. Hill and C. Tenney pers. obs.). On Chews Ridge, *S. a. clemencei* was observed nectaring on five different plant species (Fig. 4). *Cirsium occidentale* and *Monardella villosa* were the most visited flowers during both years. *C. occidentale* was used heavily within the first half of the flight season, after which the majority of the thistle on Chews Ridge senesced in 2012 and 2013. *M. villosa* blooms later in the flight season, and was used throughout the remainder of the season in 2012 and 2013. Interestingly, despite the relatively high abundance of *Asclepias eriocarpa* and *Verbena lasiostachys* in both 2012 and in 2013, *S. adiaste* adults very rarely nectared on those flowers. This indicates a preference for *C. occidentale* and *M. villosa* compared with the other three nectar sources.

Although *C. occidentale* appears to be the most commonly used nectar source, we observed between-year variation in nectar source use that points to the importance of availability of multiple nectar species for population persistence. In 2012 *C. occidentale* made up the majority of all nectaring observations (78% of 92 observations). However, in 2013, there was a shift toward *M. villosa* as the main nectar source (55% of 28 observations). The 2013 flight season was a year of low adult *S. a. clemencei* abundance on Chews Ridge, despite abundant *Viola*, probably linked to lack of rainfall (K. Zaman, C. Tenney, R. I. Hill, unpublished data). Lack of rainfall also seems to have affected the nectar source *C. occidentale* during 2013, as it senesced relatively early compared with 2012, and was unavailable as a major nectar source by mid to late June. As a result, *M. villosa* became the primary nectar source throughout the mid to late part of the flight season when females were ovipositing. Studies done with *Speyeria mormonia* have shown that fecundity declines in direct proportion to declines in adult diet (Boggs & Ross, 1993). Assuming this holds for *S. adiaste*, the lack of an alternate nectar source on Chews Ridge later in the season in drought years could severely limit the number of eggs oviposited, and thereby limit the number of overwintering larvae. This suggests that the presence of at least two nectar sources on Chews Ridge, or a preferred nectar host that persists throughout the flight season (in other localities), may be a critical factor in allowing the population to persist during drought, especially prolonged drought. Drought conditions may also have a compound effect on populations across years by causing relatively higher mortality rates among overwintering larvae, as well as reduced female fecundity in the same season, which would consequently leave fewer overwintering larvae to make it through the subsequent dry spring. Further study of ecological factors leading to population declines in this and other *Speyeria* species are clearly warranted.

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NOTE

Mosquitoes feeding on caterpillars of the Common Buckeye butterfly, *Junonia coenia* (Lepidoptera: Nymphalidae)

Mosquitoes are thought of as taking blood meals from vertebrate animals exclusively, be they mammals, birds, reptiles, or amphibians. There are even reports of mosquitoes biting fish, although at least one of them is questionable (Sloof & Marks, 1965; Mulhern, 1983). Records of mosquitoes taking meals from invertebrate hosts are far rarer, and Downes (1958) discounted records of mosquitoes feeding on invertebrate hosts. A literature search conducted via Google Scholar and the Armed Forces Pest Management Board Literature Database revealed that, since the publication of Downes' review, only a few authors have published additional accounts of mosquitoes using invertebrate hosts for blood meals (Table 1). According to Harris *et al.* (1969), invertebrate hemolymph meals taken by mosquitoes may have been missed because blood meal analyses generally are done only if the mosquito abdomen is red or dark colored, and then they are only screened against vertebrate antisera. Modern methods of blood meal identification use molecular methods but again, they are screened for vertebrate hosts (e.g. Kent & Norris, 2005). Invertebrate hemolymph generally is clear, yellow, or green; it may be mistaken for a sugar meal and ignored (Rapp, 1947; Harris *et al.*, 1969). Harris & Cooke (1969) stated that the volume of hemolymph taken from caterpillars is about the same as the volume of blood taken from vertebrate hosts; they believed that the quality of the hemolymph meal was inferior to that of the blood meal for purposes of developing eggs.

The Common Buckeye, *Junonia coenia* (Hübner), is found throughout Florida, in much of the southern United States, and into Mexico and the Caribbean (Minno & Emmel, 1993; Daniels, 2003). Three 5th instars (purchased from a butterfly farm) were placed into a cage containing about 100 female *Aedes aegypti* (L.) mosquitoes reared from larvae collected in the vicinity of Marathon, Florida. The mosquitoes were of varying ages, and had been provided a 10% sucrose solution as adults but never a blood meal. No mosquitoes attempted to feed within 10 minutes. The caterpillars were removed from the cage and "coddled" (sensu Harris *et al.*, 1969), i.e., immersed into hot water. This process was repeated three times with the same caterpillars. Every time that the coddled caterpillars were placed into the mosquito cage, numerous female mosquitoes attacked the caterpillars (Fig. 1). Attacks were almost all unsuccessful due to the caterpillars' struggles when the mosquitoes probed them, and the caterpillars' scoli appeared to obstruct attempts to feed. (It should be noted that all previous attempts to feed mosquitoes on caterpillars used larvae that were smooth and without scoli.) At least two female mosquitoes fed on the caterpillars. Both female mosquitoes that took meals from caterpillars subsequently had greenish-yellow liquid in their abdomens. One of the females was captured, photographed, and placed into a rearing chamber along with 10% sucrose and moist filter paper for oviposition (Fig. 2). Three days later the abdomen was no longer engorged. A brown stain was seen on the filter paper, suggesting the meal may have been voided, as *Ae. aegypti* are known to do; females excrete almost 40% of the liquid portion of the blood meal within the first hour after feeding (Gillett, 1956; Beyenbach, 2003). On the fifth day after feeding, the filter paper was removed and examined under a dissecting microscope. Four eggs were found (Fig. 3). The temperature of the laboratory was about 28° C, so finding eggs on the fourth or fifth day was expected (Marchoux *et al.*, 1903). The filter paper was allowed to dry and placed into a plastic bag for two weeks to allow for embryonic development and to maintain humidity. After two weeks, 24.8 ml distilled water was placed into an 8 dram glass vial and 2 mg lactalbumin was added to the water to promote bacterial growth and lower oxygen content. Bacteria serve as a food source for first instar mosquitoes; a low oxygen level in the water is a hatching stimulus for mosquito eggs. Three days were allowed for bacterial growth, after which

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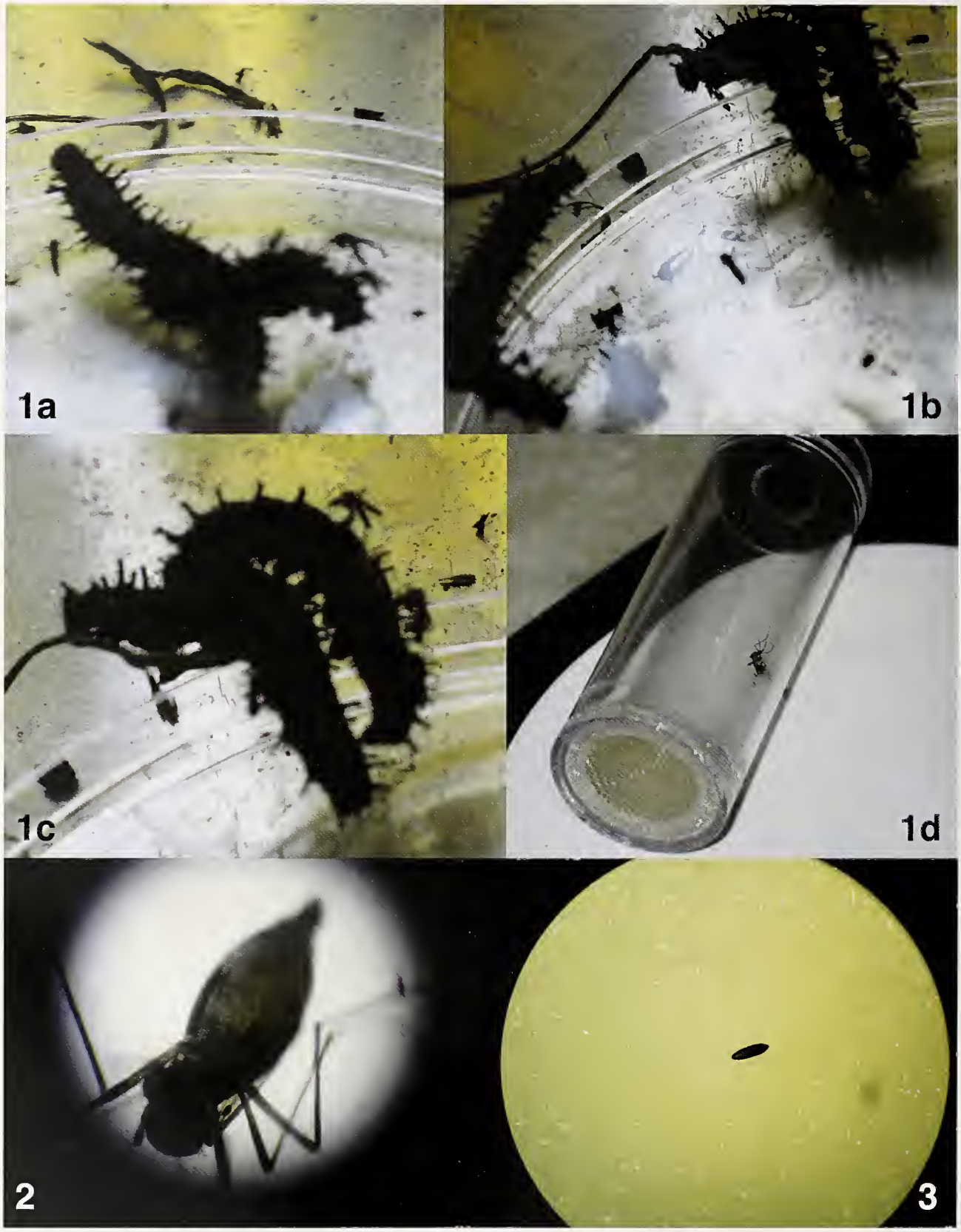

Figures 1-3. 1. Mosquitoes feeding on caterpillars (**1a, 1b, 1c**) and mosquito captured immediately after feeding (**1d**). **2**. Mosquito with hemolymph meal. **3**. One of four *Aedes aegypti* eggs laid on filter paper after the mosquito fed on a caterpillar.

Table 1. Literature records of mosquitoes feeding on caterpillars. Key: O = oviposition not reported; N = no viable eggs laid; Y = viable eggs laid.

Lepidopteran	Common Name	Mosquito	Result	Reference
Heliothis subflexa (Guenée)	Subflex Straw Moth	*Anopheles stephensi* Liston	O	George *et al.* 2013
Spodoptera littoralis Boisduval	Egyptian Cotton Leafworm	*Aedes aegypti* (L.)	O	Martel *et al.* 2011
Hyles euphorbiae (L.)	Spurge Hawk Moth	*Aedes aegypti* (L.)	Y	Harris *et al.* 1969
Hyles euphorbiae (L.)	Spurge Hawk Moth	*Culex territans* Walker	O	Harris *et al.* 1969
Hyles euphorbiae (L.)	Spurge Hawk Moth	*Culex territans* Walker	O	Harris & Cooke 1969
Manduca quinquemaculata Haworth	Five-spotted Hawk Moth	*Aedes aegypti* (L.)	N	Harris *et al.* 1969
Calophasia lunula (Hufnagel)	Toadflax Brocade Moth	*Aedes aegypti* (L.)	Y	Harris *et al.* 1969
Euxoa messoria (Harris)	Darksided Cutworm	*Aedes aegypti* (L.)	Y	Harris *et al.* 1969
Danaus plexippus (L.)	Monarch Butterfly	*Aedes aegypti* (L.)	N	Harris *et al.* 1969
Galleria mellonella (L.)	Greater Wax Moth	*Aedes aegypti* (L.)	Y	Harris *et al.* 1969

the mosquito eggs were placed into the water. Two of the eggs hatched within an hour. Four days later, a third egg had hatched. All three larvae pupated and 2 male and 1 female adult mosquitoes emerged from the pupae.

Harris *et al.* (1969) and Martel *et al.* (2011) both ask a critically important question: does this phenomenon occur in nature? If so, it could have great impacts on lepidopteran biology. Martel *et al.* (2011) reported that in the presence of mosquitoes, larvae of the noctuid moth *S. littoralis* Boisduval had a longer development time, lower pupal weight, spent less time feeding and more time trying to move away from mosquitoes than did caterpillars not exposed to mosquitoes. Time spent fleeing mosquitoes is time spent not feeding, exposure to predators is greater, and there is the risk of not finding a suitable host plant again (Martel *et al.*, 2011). There is also the unanswered question of whether mosquitoes transmit diseases to lepidopteran larvae (Martel *et al.*, 2011). George *et al.* (2013) reported that *Anopheles stephensi* Liston attacks and feeds on living and dead larvae of another species of noctuid moths, viz., *Heliothis subflexa* (Guenée). Interestingly, this mosquito is attracted to caterpillars that are infected with the fungal pathogen *Beauvaria bassiana*. Does *An. stephensi* serve as a vector of the pathogen to caterpillars? Or, do caterpillars serve as reservoirs for mosquito pathogens? Martel *et al.* (2011) reported that mosquitoes that fed on infected caterpillars themselves became infected with *B. bassiana*. In another study, all five mosquitoes that were fed on nuclear polyhedrosis-infected *H. euphorbiae* larvae died after feeding (Harris & Cooke, 1969). It may be that caterpillars serve as reservoirs for mosquito pathogens. If so, this could prove to be of importance to agriculture and public health.

Another question that remains is whether the hemolymph meal affects the mosquito that ingests it. Harris *et al.* (1969) raised the question: do toxic compounds sequestered by lepidopteran larvae interfere with mosquito egg development? Harris & Cooke (1969) observed that most mosquitoes that fed on monarch larvae died within three days. This was attributed to sequestration of toxins by the monarch butterfly larvae. Buckeye larvae sequester iridoid glycosides from their host plants (Bowers & Collinge, 1992). When present in the hemolymph, these compounds are repellant to predators (De la Fuente *et al.*, 1995).

Yet another consideration is the value of lepidopteran hemolymph as a food for mosquitoes. Harris & Cooke (1969) considered hemolymph to be an inferior food for mosquitoes, and they reported lower rates of follicular maturation and egg development. In the present instance, only four eggs were laid, whereas the usual number is between 100 and 200 eggs per vertebrate blood meal (Zettel & Kaufman, 2009).

Still one more question remains: why would mosquitoes take a hemolymph meal from caterpillars? Harris & Cooke (1969) thought that invertebrate prey could represent a food source of last resort, when no vertebrate hosts were available and eggs required a protein source for maturation. Martel *et al.* (2011) speculated that this might be one explanation, but also suggested two other possibilities. First, that under poor weather conditions mosquitoes and caterpillars might seek shelter in the same places and thus it would be easier and safer to exploit caterpillars than to leave the shelter to seek a vertebrate host. The other is that this behavior represents an ancestral feeding behavior that mosquitoes have not lost due to a lack of selective pressure against it.

In his study on the effects of mosquito control pesticides on butterfly populations in the Florida Keys, Salvato (2001) reported that, at least in some instances, butterfly numbers were greater in mosquito control-treated areas than in non-treated areas. A variety of larvicides and adulticides is used for mosquito control in Florida (Rey *et al.*, 2012). It is tempting to jump to the conclusions that mosquitoes may negatively impact caterpillars or that mosquito control operations may benefit caterpillars. The intersection of mosquito control and lepidopteran conservation is a fertile ground for investigation and controversy. It is in the hope of stimulating the former instead of the latter that the preceding observations and photographs are offered.

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A new species of the genus *Hyalophora* Duncan, 1841 from Central Mexico (Lepidoptera: Saturniidae, Attacini)

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Abstract. A new species of the genus *Hyalophora*, *H. mexicana* **sp. n.**, is described. Specimens from the Mexican Federal States of Zacatecas and Guanajuato as well as male genitalia are illustrated; a distribution map is included. The male holotype is deposited in Colección Nacional de Insectos, Universidad Nacional Autónoma de México, Ciudad México, Mexico. Only male specimens are known so far. The new species is compared with other *Hyalophora* species. The description of the new species is based on studies of imaginal morphology including male genitalia and mtDNA (COI barcode). *H. mexicana* **sp. n.** is a very large species for the genus, and with its combination of typical characters such as reddish colouration, rounded, drop-shaped ocellular patches of the wings, a reduced, quarter-circle band of blue scales in the subapical ocellus of the forewing and details in male genitalia structures it can be separated easily from all hitherto known northern species. In addition, some taxonomic and nomenclatural problems in *Hyalophora* are addressed.

Key words: *Hyalophora mexicana* **sp. n.**, Zacatecas, Guanajuato, Sierra Madre Occidental.

Introduction

The genus *Hyalophora* Duncan, 1841 is presently known with three species from the North American continent, especially from north of Mexico. According to recent literature (e.g., Lemaire, 1978; 1996; Tuskes *et al.*, 1996; Collins, 1997; Powell & Opler, 2009), the following three species are known from southern Canada and the United States:

Hyalophora cecropia (Linnaeus, 1758) in the eastern and central area (western limits see Peigler & Opler, 1993; the maps by Peigler & Opler were made before the true nature of *kasloensis* was published and contain misidentifications; their plots for *cecropia* in Utah and Washington are almost certainly errors; Collins, pers. comm.),

H. columbia (Smith, 1865) with two (or three) subspecies, *H. columbia columbia* (and perhaps *H. c. nokomis* (Brodie, 1894)?) in central to eastern Canada and *H. c. gloveri* (Strecker, 1872) in the Rocky Mountains/ Great Basin area; this taxon was listed as a separate fourth species by Ferguson, 1972), with a red "form" in southern Arizona (Ferguson, 1972: 257; Peigler & Opler, 1993; Powell & Opler, 2009: 241); and

H. euryalus (Boisduval, 1855) [= *rubra* Neumoegen & Dyar, 1894] in the West, primarily along the Pacific Coast (Peigler & Opler, 1993; Tuskes *et al.*, 1996), with probably a subspecies on Cedros Island, Baja California, Mexico (see below). Populations in the interior of British Columbia, Canada, and in the Bitterroot Mts. of Idaho and Montana (USA), named as subspecies *kasloensis* Cockerell [*in* Packard, 1914], have usually been treated as a hybrid intergrade between *euryalus* and *gloveri* (Sweadner, 1937; Tuskes *et al.*, 1996; Collins, 1997; 2006), or alternatively as a subspecies of *euryalus* (e.g., Ferguson, 1972).

* 83rd Contribution to the Knowledge of the Saturniidae (82nd Contribution see: Naumann, S., S. Löffler & W. A. Nässig. 2012. Revisional notes on the species-group of *Saturnia cachara*, with description of a new subgenus and a new species (Lepidoptera: Saturniidae). — Nachrichten des Entomologischen Vereins Apollo, Frankfurt am Main, N.F. **33** (2/3): 107–128).

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These three species are closely related, and most authors agree that local hybridisation and introgression occurs in areas where they meet naturally (Tuskes *et al.,* 1996; Collins, 1997; 2007; Collins & Rawlins, 2014). Apparently *H. columbia/gloveri* and *H. euryalus* hybridize everywhere they are sympatric or parapatric (Collins, 1984; 1997). Nominotypical *H. columbia* and *gloveri* appear to intergrade completely across Canada (Collins, 1984; Kohalmi & Moens, 1975; 1988). Only *H. cecropia* apparently maintains integrity in sympatry with congeners (Collins, pers. comm.). Earlier observations that at some other places the distribution areas may possibly overlap without regular hybridisation (e.g., Tuttle, 1985; Tuskes *et al.,* 1996) have not found support. In the laboratory obviously all populations can readily be hybridised, but often with at least partially reduced fertility of the hybrid offspring (Tuskes *et al.,* 1996; Collins, 1997). In the wild, a possible hybrid offspring of presently local, isolated populations caused by climate fluctuations and resulting distribution area shifts during especially the ice ages, in which two taxa came into contact only temporarily, may be obscured, and only sometimes can be demonstrated by introgression (Collins, pers. comm.). Further, there are small, but apparently stable differences in male genitalia morphology (Ferguson, 1972; Lemaire, 1978), besides obvious differences in external adult and preimaginal morphology (e.g., Tuskes *et al.,* 1996; Lampe, 2010).

Published records of *Hyalophora* for Mexico are sparse and mostly cover the northern states of Mexico bordering the U.S. only (Fig. 1):

H. euryalus in Baja California Norte, including Cedros Island on the Pacific side of the Baja California peninsula. A separate subspecies was described from this island: *cedrosensis* Cockerell [*in* Packard], 1914; this is — according to literature — the southernmost population of *H. euryalus* (Hoffmann, 1942; Ferguson, 1972; Lemaire, 1978; Smith & Wells, 1993; Tuskes *et al.,* 1996; Powell & Opler, 2009). Material from Baja California and Cedros Island was not accessible to us for this study.

A large reddish form occurs in the northern Mexican federal states of Chihuahua and Sonora (Peigler, 1994; Tuskes *et al.,* 1996) close to the US border, and also in Durango and "to an undetermined distance south" (Tuskes *et al.,* 1996: 208) within the Sierra Madre Occidental, always interpreted as belonging to *H. columbia gloveri,* according to Tuskes *et al.* (1996).

Beutelsbacher-Baigts & Balcázar-Lara (1994: 19) speculated that *H. cecropia* might live in the north of the state of Tamaulipas close to the Texas border, but no record was provided.

Published records from further south in Mexico are even rarer and appear to be somewhat questionable: Hoffmann (1942) reported "*Platysamia cecropia*" from Veracruz state (near Jalapa; recent spelling: Xalapa), but interpreted it as an introduced population; however, Tuskes *et al.* (1996) suspected that this record may also refer to what they call an "especially large, reddish form of *gloveri.*" We have not seen any specimen from Veracruz so far. The specimen cited by Hoffmann (1942) has not been located in his collection today deposited in Ciudad México (Colección Nacional de Insectos, Universidad Nacional Autonóma de México) during a search by one of the authors (G.N.G.). *H. cecropia* is exclusively associated with temperate North American open woodland and meadows, especially somewhat disturbed areas (even city suburbs); apparently, it reaches its warm climate tolerance near San Antonio (Texas) (Collins, pers. comm.). However, Ferguson (1972: 247) also listed it from Brownsville (Texas) at the Rio Grande borderline between the USA and Mexico. So, if it is not just a misidentification of a population of "*H. gloveri* sensu lato" (Tuskes *et al.* 1996) or the result of an artificial introduction of *H. cecropia* as indicated by Hoffmann (1942), it may alternatively reflect a formerly continuous distribution of *H. cecropia* through the lowlands and lower slopes of the Sierra Madre Oriental along the Caribbean (eastern) coastline of Mexico. Brownsville in Texas at the Rio Grande is, in a bent line along the Caribbean coastline, approximately 750 km from Xalapa in Veracruz state, and a much smaller distance from Tamaulipas (Beutelsbacher-Baigts & Balcázar-Lara, 1994, but see above).

Specimens found in the Sierra Madre Occidental range in the Mexican federal states of Guanajuato and Zacatecas by one of the authors (G.N.G.) between 1997 and 2013 gave us the chance to study these apparently rare Central Mexican moths in more detail. A few further specimens of this population were found by G.N.G. and Jean Haxaire, also in Zacatecas (now in collections D. Herbin and R. Rougerie). Every colleague with whom we discussed these findings in early times of our study expected (pers. comm.), according to Tuskes *et al.* (1996), that the Central Mexican red *Hyalophora* would be southern outliers of those northern red populations in Arizona and northern Mexico (Sonora, Chihuahua, Durango).

The mtDNA barcodes of several of these Guanajuato and Zacatecas specimens were analysed. During our study we compared our data with other *Hyalophora* barcodes, either of our own material or in public records in BOLD, and also with further data through support by Rodolphe Rougerie as well as Michael M. Collins, Jim Fetzner and John Rawlins

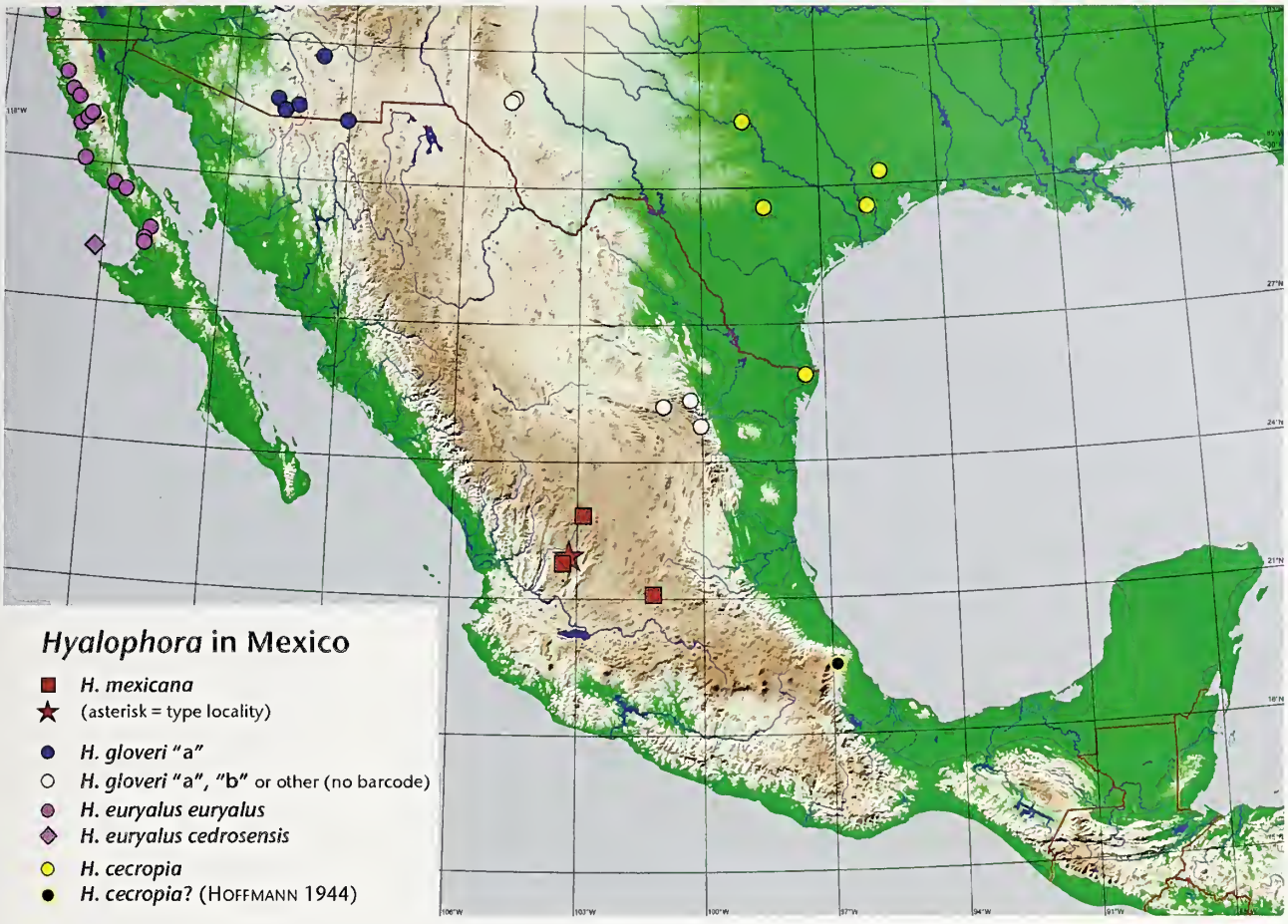


Figure 1. Distribution of *Hyalophora mexicana* and related species of *Hyalophora* in Mexico and bordering states of the USA (species code see insertion). One dot may represent more than one locality if in close proximity; we have not located every label or published data on the map. Many more or less inexact records of (usually) "*H. columbia gloveri*" from northern Mexico and southern USA in general literature have not been included or (when exact locality data was available) are represented as white dots, as they have not been identified by barcode. — Map created with Map Creator 2.0 Personal Edition, © 2003–2007 primap software, modified and localities added (W.A.N.).

(Carnegie Museum of Natural History, Pittsburgh, PA). In spite of the fact that there still are some incertainties (mainly due to lack of data) about several of the northern taxa and populations of *Hyalophora*, we decided to describe this southernmost, now relatively well-characterised population from Guanajuato and Zacatecas here according to the results of this study as a new species of the genus, based on external and genitalia morphology and sequence data of part of the mitochondrial COI gene.

There are still taxonomic problems. Apparently not every population in the USA which presently is called "*Hyalophora columbia gloveri*" truely represents that species (respectively, subspecies), based on our present knowledge. A paratype of the new species will be deposited in the Carnegie Museum to allow American colleagues access to the new species.

Materials and methods

We used all *Hyalophora* specimens available to us in the collections of Senckenberg Frankfurt (SMFL), coll. S. Naumann, Berlin, and, for the new species, all specimens collected by G.N.G. and later dispersed to the collections shown in the types list. Morphological studies on imagos followed standard procedures; photos of the set specimens were taken with a digital camera and a circular daylight fluorescent tube. The last segments of the abdomen of male moths were cut off and macerated in ca. 2–3% aqueous NaOH solution at ca. 96–98°C for 1 h to clean the genitalia from scales, fat and tissue. After dissection in water and low-concentrated ethanol, the genitalia were stored in 70% ethanol in vials. The drawings were produced from "unflattened" genitalia in ethanol;

we believe, in accordance with, e.g., Zwick (2009: 148), that the preservation of the undistorted three-dimensional structure of the genitalia is essential for the understanding of their function. The genitalia photograph was taken from a flattened, slide-mounted preparation.

Data of the specimens which were used for the mtDNA analysis are listed in Table 1. The so-called COI barcode is based on the sequence data of a short 658 base pairs region of the mitochondrial cytochrome-*c* oxidase, subunit I [COI], gene. DNA was extracted from the legs of dried specimens mainly in the collections of the authors. Further sequence data kindly provided by Michael M. Collins, Jim Fetzner and John Rawlins were used for comparison. Technical details and references relative to the laboratory protocols see in Ratnasingham & Hebert (2007), on the CCDB website (CCDB 2014) and also in, e.g., Decaëns & Rougerie (2008) or Vaglia *et al.* (2008). Sequences of the specimens analysed are deposited in GenBank (Table 1). The analysis of sequence data was conducted using MEGA5 (Tamura *et al.*, 2011); see Fig. 2.

We used one specimen each of the three species of the genus *Callosamia* as outgroup to root the mtDNA barcode trees. *Callosamia* generally most often shows up as North American sistergroup of *Hyalophora* in COI barcode trees, irrespective which analysis method is used.

The COI barcode data of 37 *Hyalophora* plus the 3 *Callosamia* specimens used in our analysis were generated either in Guelph, Ontario, by Bold (2014) or provided by Jim Fetzner, John Rawlins and Michael M. Collins. We used only sequences that were more than 600 base pairs (bp) long. Shorter sequences as well as those from specimens of doubtful origin were discarded.

Abbreviations used

BC — Barcode [no.].
CDHP — Collection Daniel Herbin, Pechabou, France.
CGNG — Collection Guillermo Nogueira G., Zapopan, Jalisco, México.
CMNH — Carnegie Museum of Natural History, Pittsburgh, PA, USA.
INBUNAM — Instituto de Biología (Colección Nacional de Insectos), Universidad Nacional Autónoma de México, Ciudad México (Mexico City), Mexico.
CRRR — Collection Rodolphe Rougerie, Rouen, France.
CSNB — Collection Stefan Naumann, Berlin, Germany.
SMFL — Senckenberg-Museum, Lepidoptera collection, Frankfurt am Main, Germany (including coll. W. A. Nässig).
UAG — Universidad Autónoma de Guadalajara collection, Zapopan, Jalisco, México.

Results

Based on our results, we here describe the "big red species" of *Hyalophora* from Central Mexico (Mexican Federal States of Zacatecas and Guanajuato) as a new species:

***Hyalophora mexicana* sp. n.**

Holotype ♂: Mexico, Zacatecas, La Manchada, 1966 m, 28. VII. 2003, dissection number WAN 1988/07, barcode B3218-wn-B08, leg. G.N.G. Deposited in the INBUNAM collection at the Universidad Nacional Autónoma de México in Mexico City, Mexico. Figs. 3a–3b.

Paratypes (in total 10 ♂♂), all Mexico (see Fig. 1, map):

Guanajuato (3 ♂♂): 1 ♂, Sierra de Santa Rosa, 2300 m, 2.–3. VII. 1997, leg. G.N.G., Barcode B3218-wn-B07, SMFL (Figs. 4a–4b). 1 ♂, Sierra de Santa Rosa, 2634 m, 8. VII. 2013, leg. G.N.G., CGNG. 1 ♂, Sierra de Santa Rosa, 2347 m, 9. VII. 2013, leg. G.N.G., CSNB.

Zacatecas (7 ♂♂): 1 ♂, Florencia de B.J., 2114 m, 26. VII. 2003, leg. G.N.G., SMFL. 2 ♂♂, Tlaltenango de Sánchez, 2591 m, 27. VII. 2003, leg. G.N.G., barcode SNB 1686 (this specimen in CSNB, the other specimen will be deposited in CMNH). 1 ♂, La Manchada, 1966 m, 28. VII. 2003, dissection number WAN 1965/05, barcode B3218-wn-B09, UAG. 1 ♂, La Manchada, Momax, 1950 m, 21°57'54" N, 103°12'20" W, 17. VI. 2009, SMFL. — 1 ♂, Zacatecas, 'dirt road' Momax to San Lorenzo, behind San Lorenzo, 2 km toward La Manchada, 1935 m, 30. VII. 2003, leg. G.N.G., J. Haxaire & O. Paquit, BC-Roug1230, CRRR. 1 ♂, Zacatecas, same data, but 28. VII. 2003, BC-Her2360, CDHP.

Etymology: named after the country of origin, Mexico.

Description and diagnosis

♂: Generally, a large species in the genus, with the holotype in most measurements being the largest and the specimens from Guanajuato on average the smallest.

As given in detail below, it is unique in the genus by the combination of its reddish-brown ground colour; the quite rounded, drop-shaped ocellular patches of both fore- and hindwings; a typical blue marking of the forewing subapical ocellus; broad and relatively straight, not undulate postmedian lines; and in male genitalia the extended sacculus and the typical spine of the vesica.

Measurements: Holotype: forewing: length (fwl., measured from base to tip of apex) [all wing measurements of left side of holotype] 7.8 cm, forewing (fw.) eyespot or discoidal patch largest diameter (l.d.) 1.3 cm, apical spot l.d. 0.86 cm; hindwing length (hwl.) 5.6 cm, hw. eyespot l.d. 1.4 cm. Antenna ca. 20.3 mm long, longest rami ca. 4.0 mm long. — All males (holotype and available paratypes together, $n = 7$ except see below): fwl. 6.4–7.8 cm, average 6.89 cm ± 0.46 s.d.; fw. eyespot l.d. 1.1–1.3 cm, average 1.19 cm ± 0.08 s.d.; apical spot l.d. 0.60–0.86 cm, average 0.67 ± 0.08 s.d.; hindwing length (hwl.) 4.7–5.6 cm, average 5.17 cm ± 0.29 s.d.; hw. eyespot

Table 1. Data of the specimens of *Hyalophora* (37 specimens) and *Callosamia* (3 specimens, included as outgroup) used for the mtDNA sequence analysis. — Additional abbreviations: GBAC = GenBank Access Code; HT = holotype; PT = paratype; SL = Sequence Length (data from BOLD or simple count of bp); — = information not available. — In the same order of taxa and specimens as in the tree graph, Fig. 2.

Species	Sample-ID	Process-ID	GBAC	SL	Deposition	Locality of Origin
H. gloveri "a"	SNB 1699	SASNB699-09	GU702999	658[0n]bp	CSNB	USA, Arizona, Cochise Co., Guadelupe Canyon
H. gloveri "a"	SNB 1863	SASNB768-10	HQ579817	658[0n]bp	CSNB	USA, Arizona, Cochise Co., Guadelupe Canyon
H. gloveri "a"	MGS 734	—	KJ865746	658 bp	CMNH	USA, Arizona, Cochise Co., Huachuca Mtns.
H. gloveri "a"	MGS 723	—	KJ865745	658 bp	CMNH	USA, Arizona, Cochise Co., Huachuca Mtns.
H. gloveri "a"	MGS 721	—	KJ865744	658 bp	CMNH	USA, Arizona, Cochise Co., Huachuca Mtns.
H. gloveri "a"	MGS 707	—	KJ865743	658 bp	CMNH	USA, Arizona, Graham Co., Pinaleno Mtns.
H. gloveri "a"	MGS 569 = 570	—	KJ865742	658 bp	CMNH	USA, Arizona, Hualapai
H. gloveri "a"	MGS 233	—	KJ865741	658 bp	CMNH	USA, Arizona, Gila Co., Payson
H. columbia (grey like *gloveri*)	SNB 1861	SASNB766-10	KM287184	658[0n]bp	CSNB	USA, Colorado, Colorado Springs
H. columbia (blackish)	B3218-wn-C05	SAWNA027-09	GU703464	658[0n]bp	SMFL	Canada, Ontario, Norland
H. columbia (blackish)	B3218-wn-C06	SAWNA028-09	GU703465	658[0n]bp	SMFL	Canada, Ontario, Norland
H. columbia (grey like *gloveri*)	SNB 1860	SASNB765-10	HQ579815	658[0n]bp	CSNB	USA, Colorado, Colorado Springs
H. columbia	SNB 1698	SASNB698-09	HM383529	658[0n]bp	CSNB	Canada, Ontario, Haliburton Highlands
H. columbia	SNB 1857	SASNB762-10	HQ579814	658[0n]bp	CSNB	Canada, Ontario
H. columbia (hybr. with *cecropia*?)	SNB 1866	SASNB771-10	HQ579818	658[1n]bp	CSNB	Canada, Ontario, Haliburton Highlands
H. cecropia	SNB 3227	SASNC1238-11	KM287185	658[0n]bp	CSNB	USA, New Jersey, Beachwood
H. cecropia	SNB 1869	SASNB774-10	HQ579819	658[0n]bp	CSNB	USA, Texas, Bexar Co., San Antonio
H. cecropia	SNB 3237	SASNC1248-11	KM287195	658[0n]bp	CSNB	USA, Wisconsin, Portage Co.
H. columbia nokomis (hybr. with *cecropia*?)	SNB 1859	SASNB764-10	KM287192	658[0n]bp	CSNB	Canada, Ontario, Mafeking
H. cecropia	B3218-wn-B11	SAWNA022-09	GU703463	658[0n]bp	SMFL	Canada
H. cecropia	SNB 3228	SASNC1239-11	KM287183	658[0n]bp	CSNB	USA, New Jersey, Beachwood
H. cecropia	SNB 1867	SASNB772-10	KM287193	658[0n]bp	CSNB	Canada, Ontario, Haliburton Highlands
H. cecropia	SNB 1871	SASNB776-10	KM287190	658[0n]bp	CSNB	USA, Colorado, Denver
H. euryalus	B3218-wn-C02	SAWNA024-09	GU703536	658[0n]bp	SMFL	USA, California, Monterey
H. euryalus	SNB 1694	SASNB694-09	GU703001	658[0n]bp	CSNB	USA, California, San Diego, vic. Escondido
H. euryalus	B3218-wn-C01	SAWNA023-09	GU703535	658[0n]bp	SMFL	USA, Washington, Chelan Co., vic. Leavenworth
H. euryalus kasloensis	SNB 1696	SASNB696-09	GU703000	658[0n]bp	CSNB	Canada, Brit. Columbia, Okanagan Valley
H. euryalus	SNB 1695	SASNB695-09	GU703002	658[0n]bp	CSNB	USA, California, Nevada Co.
H. euryalus	SNB 1858	SASNB763-10	KM287188	658[0n]bp	CSNB	USA, California, Nevada Co.
H. gloveri "b"	B3218-wn-C03	SAWNA025-09	GU703533	658[0n]bp	SMFL	USA, Utah, Box Elder Co.
H. gloveri "b"	B3218-wn-C04	SAWNA026-09	GU703534	658[0n]bp	SMFL	USA, [reared, no data]
H. gloveri "b"	SNB 1864	SASNB769-10	KM287189	658[0n]bp	CSNB	USA, Utah
H. mexicana **PT**	SNB 1686	SASNB686-09	GU703009	658[0n]bp	CSNB	Mexico, Zacatecas, Tlaltenango de Sánchez
H. mexicana **PT**	B3218-wn-B07	SAWNA018-09	GU703460	658[0n]bp	SMFL	Mexico, Guanajuato, Sierra de Santa Rosa
H. mexicana **HT**	B3218-wn-B08	SAWNA019-09	GU703461	658[0n]bp	SMFL	Mexico, Zacatecas, La Manchada
H. mexicana **PT**	B3218-wn-B09	SAWNA020-09	GU703462	658[0n]bp	SMFL	Mexico, Zacatecas, La Manchada
H. mexicana **PT**	BC-Roug1230	SATWB181-11	KM287191	633[0n]bp	CRRR	Mexico, Zacatecas, 'dirt road' Momax to San Lorenzo, after San Lorenzo
C. promethea	SNB 1856	SASNB761-10	HQ579813	658[0n]bp	CSNB	Canada, Quebec
C. angulifera	SNB 1853	SASNB758-10	HQ579812	658[0n]bp	CSNB	USA, Pennsylvania
C. securifera	SNB 1852	SASNB757-10	KM287187	633[1n]bp	CSNB	USA, Florida, Lake Co.

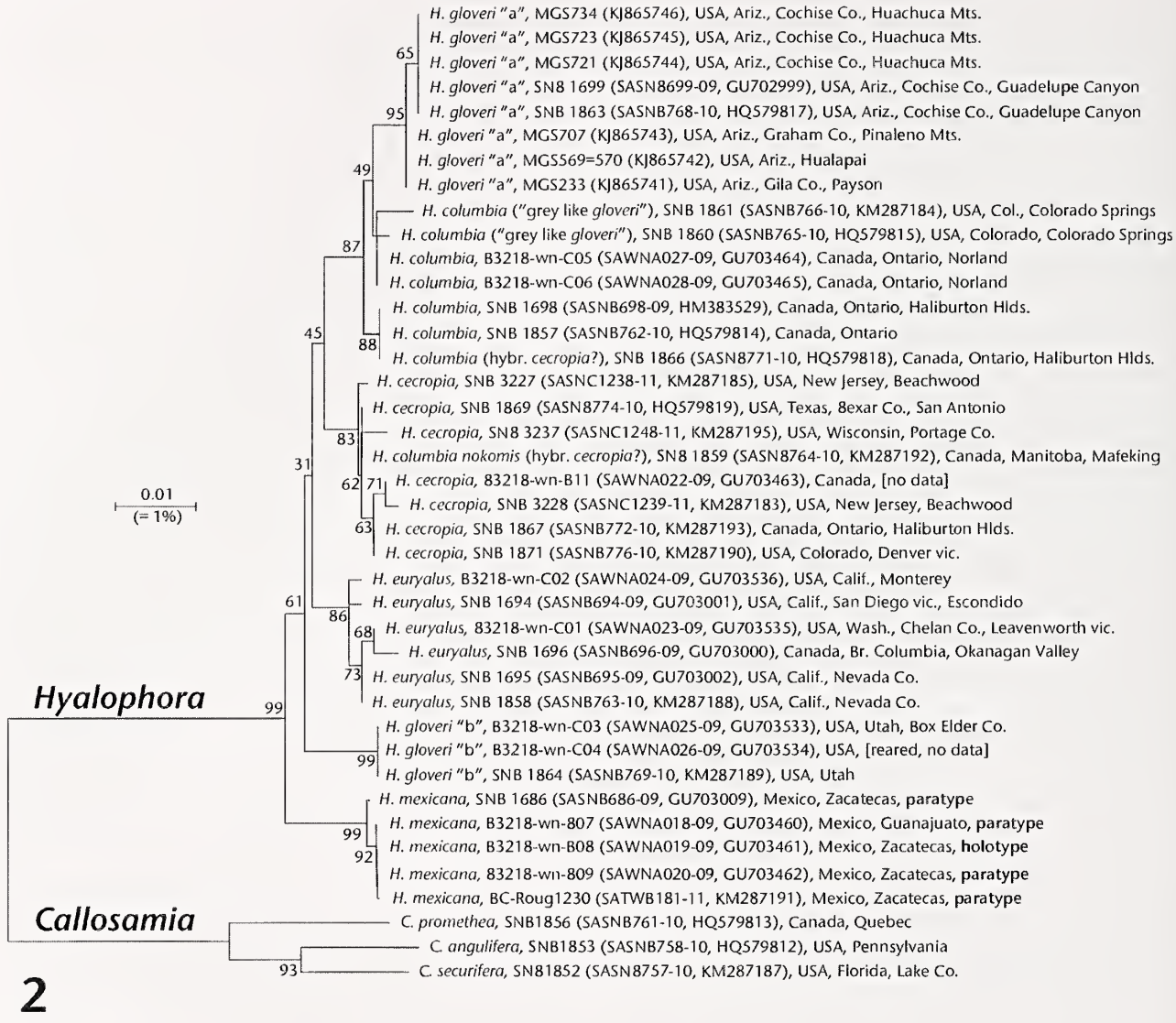


Figure 2. The tree of *Hyalophora* taxa was inferred using the Neighbour-Joining method (Saitou & Nei, 1987). The optimal tree with the sum of branch length = 0.19203706 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (3000 replicates) are shown next to the branches (Felsenstein, 1985). Evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.*, 2004). The rate variation among sites was modeled with a gamma distribution (shape parameter = 3). The differences in the composition bias among sequences were considered in evolutionary comparisons (Tamura & Kumar, 2004). The analysis involved 40 nucleotide sequences (= specimens). There were a total of 653 positions in the final dataset. Analyses were conducted in MEGA5 (Tamura *et al.*, 2011).

l.d. 1.0–1.5 cm, average 1.29 ± 0.14 s.d. Antenna with ca. 33–34 segments (n = 2), quadripectinate to their tip; ca. 17–21 mm long (n = 7, average 19.19 mm ± 0.13 s.d.), longest rami ca. 3.5–4.0 mm (n = 7, average 3.79 mm ± 0.21 s.d.).

Colour and wing pattern: Ground colour on dorsal side intensive reddish brown; colour tone similar to *H. euryalus*. Antennae very dark brown to mostly black (in the similar *H. euryalus* dark brown). Head, dorsal parts of thorax and abdomen in ground colour, between head and thorax a wide white collar, thorax and abdomen separated by a white band of long hair, abdomen with intersegmental white stripes.

Forewing in ground colour, with broad white antemedian and little bent postmedian line, both bordered black to median area, nearly straight and not dentate, white middle part widening to the anal margin. The forewing ocellular patch more or less rounded, drop-shaped, with its tip directed to the outer margin, white, with yellow, orange and black

outer border. Postmedian area again in ground colour, in the marginal parts suffused with ochreous scales, then becoming completely ochreous. Marginal area separated from the postmedian area by a thin black line with rounded indentions between the veins, of light ochreous-white colour with around 2 mm broad darker outer margin (in most of the northern taxa, especially in the generally similar *H. euryalus*, this median area is darker greyish, not differing in tone from outer postmedian area). The apical postmedian area with a violet and pink dash, and an outer small white zigzag line. The round subapical ocellus intensive black with some blue scales in shape of a quarter circle in the top and inner quadrant of the subapical ocellus (in most specimens of the other taxa this bluish "eye shadow" forms a full lunule in the inner half of the ocellus).

Hindwing of same colouration as forewing, but basal/antemedian area whitish, without red. The hindwing discoidal patch a somehow drop-like widened lunule, with its tip directed to the outer margin. Along the white postmedian line on outer side the inner portion (for about 1 mm) of the postmedian area only with red, not interspersed with grey or black scales, somehow resembling *H. cecropia* in this aspect (in other taxa, the grey to black scales are interspersed just to the white postmedian), but without orange colour tone. The ochreous part of the postmedian area shows a row of grey patches (in most other taxa darker grey or black). On ventral side with almost same ornamentation, but of much darker colour, much less red. Thorax with legs and the abdomen in ground colour. Both fore- and hindwings are strongly suffused with black and white scales, and only the marginal postmedian area and the marginal area are of same colour as on upperside. The antemedian line is completely missing on all wings, and the hindwing has a broad white upper margin. The underside is somewhat similar to *H. euryalus;* other species with much less red on underside, mostly grey.

♂ **genitalia** (Fig. 6, Fig. 7; Figs. 8–10 for comparison with several other *Hyalophora* taxa): Uncus bifid with two long rounded tips, curved to ventral side. Valves of *Hyalophora* are generally quite globularly in shape and impossible to be flattened without distortion for photographing. Dorsal process of the valves somewhat rounded, bent to inner side, the ventral one small, slender, and with a ventrally pointing tip. The sacculus is well developed, with rounded tip, and larger than in any other *Hyalophora* species. Gnathos narrowing to tip, sometimes with a little indention (in other species always broader to tip, more plate-like), juxta with two lateral short symmetrical triangular processes, slightly shorter than in other taxa. Saccus large and rounded. Phallus straight, sclerotised part ca. 4.8–5 mm long, vesica with a dorsal projection with sclerotised thorn, a thorn-like unsclerotised ventral projection, and a distal longer bulb.

♀, **preimaginal instars, ecology and larval foodplants** unknown.

General information on and description of some of the collecting localities and methods

Zacatecas, La Manchada: Elevation 1966 m, 21°57' N, 103°12' W; 28. VII. 2003; 1950 m, 21°58' N, 103°12' W, 17. VII. 2009, Momax. The area in Zacatecas where most of the *Hyalophora* specimens were collected is found at the "Region Mezoamericana de Montaña" in the "Province of the Sierra Madre Occidental" (Rzedowski, 1994), with sedimentary and volcano-sedimentary rocks from the Cenozonic Era (INEGI 1981) with a moderately acidic soil. A locality is found in a small canyon that runs down from E to W from the Sierra Morones. The climate classification at the area according to Garcia (1973) is "Acwo (semicalido y subhumedo)." The area has about 700–1000 mm annual rainfall, 60–65% of average relative humidity and an annual mean temperature range of 18–22° C (Llorente *et al.*, 1996). At the collecting site we found a *Quercus* forest (Fig. 11), mixed with some other vegetation elements. Plant species in the forest vegetation recorded for this area included: *Quercus chihuahuensis* (Trel.), *Qu. eduardii* (Trel.), *Qu. magnoliifolia* (Née) (Fagaceae), some *Pinus oocarpa* (Schiede), *P. michoacana* (Martínez) (Pinaceae), some other vegetation elements from lower and higher altitude range present at this site are *Acacia schaffneri* (S. Watson), *A. farnesiana* (L.) Willd., *Prosopis* spp., *Mimosa* spp., *Mimosa biuncifera* (Benth.) (Fabaceae), *Larrea* spp. (Zygophyllaceae), *Baccharis* spp. (Asteraceae), *Opuntia* spp. (Cactaceae), *Arbutus arizonica* (Gray) Sarg. (Ericaceae) (Rzedowsky 1994, Vazquez-Garcia *et al.* 2004). The ♂♂ of *Hyalophora mexicana* n. sp. arrived to lights between 21:30 h and 0:30 h, after this time the weather often became very windy. Nevertheless we continued ligh-trapping until 3:00 h or later, waiting for a ♀, but none appeared. These activity times are slightly in contrast to the observations by Collins (2007: 69) on *Hyalophora* males arriving at light around 4:00 h.

Guanajuato, Sierra de Santa Rosa: Elevation 2300 m, 21°5' N, 101°12' W; 2.–3. VII. 1997. This locality which provided our first record for the new *Hyalophora* is found in the "Region Xerofitica Mexicana" in the "Province of the Altiplanicie" (Rzedowski, 1994), with extusive igneous rocks of the Cenozoic Era (INEGI 1981) with moderately acidic

soils. The locality is found in a canyon that runs down from SE to NW. The climate classification at the area according to García (1973) is "Acwo (semicalido y subhumedo)." The area has about 700–1200 mm annual rainfall, 60–65% of average relative humidity and an annual mean temperature range of 18–22° C (Llorente *et al.*, 1996). At the collecting site we found a *Quercus* forest, mixed with some other elements. Plant species in the forest vegetation recorded for this area at higher altitudes included *Pinus oocarpa* (Schiede), *P. michoacana* (Martínez) (Pinaceae), at the collecting site we found *Quercus crassifolia* (Humb. & Bonpl.), *Quercus mexicana* (Humb. & Bonpl.), *Qu. jaralensis* (Trel.), *Qu. castanea* (Née), *Qu. rugosa* (Née) (Fagaceae); some other elements are *Salix* spp., *Populus* spp. (Salicaceae), *Fraxinus* spp. (Oleaceae), *Cercocarpus* spp. (Rosaceae) (Rzedowsky 1994, Zavala 2003). The first ♂ found at this locality arrived to the lights between 22:30 h and 23:30 h.

Collecting methods in all localities: Regularly two different sets of lights were operated in parallel by G.N.G. who collected most of the specimens known. For one set of lights two white sheets were used, one in vertical position and the second on the floor; one 20 W UV light tube with filter ("superactinic light"), one 20 W UV light tube without filter ("blacklight"), two 15 W UV tubes with filter and a 150 W Mercury vapor bulb with white and UV light, all powered by a generator. For the second set of lights, again two small white sheets were used, one placed on the floor and the second at ca. 80 cm above on a tripod. This set was placed in the forest approx. 100 m away from the first set (powered by the generator or by a battery) and consisted only of originally two (later three) 20 W UV light tubes, one with filter and the other one(s) without filter. This smaller set of UV tubes is internally called the "ghost", because it looks very strange at dark night in the wild from some distance. Regularly the collecting time started at the beginning of the night and ended at sunrise. Most of the *Hyalophora* specimens arrived at this smaller set; the 2009 specimens at the larger equipment with (at that time) 3 tubes.

Potential larval hostplants: Preimaginals are thus far unknown. Known hostplants for other *Hyalophora* species in the wild (south of Canada) usually comprise members of Rosaceae, Ericaceae and Rhamnaceae families (besides a few others). With respect to the Sierra de Santa Rosa, Martínez-Cruz *et al.* (2009) list *Rosa* spp. (Rosaceae), *Ceanothus coeruleus* (Lag.) (Rhamnaceae) and, quite common, *Arbutus glandulosa* (Mart. & Gal.) (Ericaceae) to be found in this area. In fact, at least one bush of *Ceanothus coeruleus* was very close to the "ghost" light collecting site in the Sierra de Santa Rosa. So, potential standard foodplants of the genus appear to be well available in the area.

Discussion

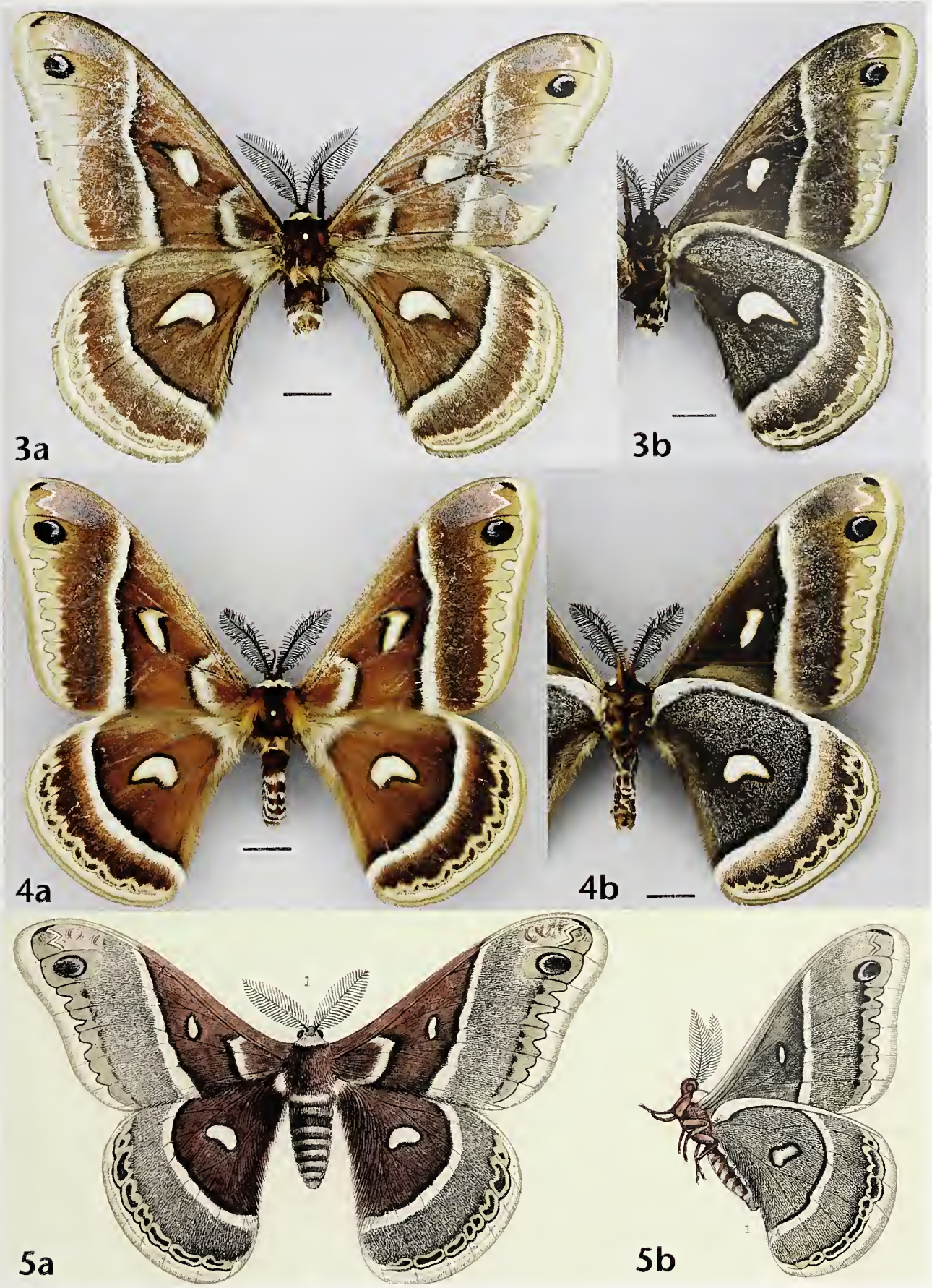
Definition and distribution of the new species *H. mexicana*

An interesting result of our study was that the red "*gloveri*" from southeastern Arizona and northern parts of Mexico close to the US border is specifically distinct from the red Central Mexican *H. mexicana* sp. n. (and possibly also from other, usually more greyish "*gloveri*" populations from USA). This result was in contrast to interpretations and predictions by earlier authors and colleagues.

Presently we do not know much about geographical distinction and ecological differences between the complex of "*H. c. gloveri* sensu lato" and the new species *H. mexicana* within Mexico and especially the Sierra Madre Occidental range. The northern and central parts of Mexico and of the Sierra Madre Occidental range are mostly dry and desert-like (e.g., the large Sonora Desert). We suppose that the populations in the southern Sierra Madre Occidental range (in Zacatecas, Guanajuato and possibly elsewhere?) are presently rather well isolated from the northeastern populations (i.e. those from localities in Coahuila and Nuevo León in the Sierra Madre Oriental, see map), as well as from the northwestern populations (i.e. those from the localities in northern Durango, Sonora and Chihuahua). These northwestern populations indeed appear to be southern outliers of the different "*gloveri sensu lato*" populations of New Mexico, Arizona, and western Texas (Tuskes *et al.*, 1996) or separate populations (or "haplotype groups") with more or less differentiated gene pools.

Morphological differences between the long accepted three (or more) northern species of the genus *Hyalophora* are easily visible, mainly in the different colouration of the involved species; differences in male genitalia are generally minor and mainly based on the form of the processes of the uncus, the ventral process of the valves, the sacculus, and the size and number of spines on the vesica. The here described *H. mexicana* has almost the same reddish-brown

Figures 3–5 (Opposite page). Specimens of *Hyalophora*; a = uppersides, b = undersides. **3.** *H. mexicana*, holotype ♂. **4.** *H. mexicana*, paratype ♂, Guanajuato. Scale bars = 1 cm. **5.** *Platysamia gloveri* Strecker, 1872, ♂ syntype; copied from Strecker's (1872) plate, fig. 1. No scale.


3a
3b
4a
4b
5a
5b

ground colour as *H. euryalus*, but is larger than this species and has more rounded, somewhat drop-like discoidal patches. *H. columbia* s.l., when reddish in ground colour at all, shows a more purple, carmine or darker blackish colour. *H. cecropia* has a typical orangy red colour element in the postmedian fascia which is not shared by any other taxon except (but there without any orange tone) in *H. mexicana*. In ♂ genitalia the two sclerotised spine-like projections of the vesica are only shared with *H. columbia* s.l., and the somewhat prominent sacculus is also found, clearly less pronounced, in *H. euryalus*.

COI barcode data

Not unexpectedly, the taxa of the genus *Hyalophora* appear to be closely related, and their speciation process is obviously rather recent and perhaps not yet "finished" in all cases. They are clearly "good" species in terms of wing pattern, immature characters, ecology, and geographic distribution (Collins, pers. comm.). The differences in the barcode sequences are rather low (Fig. 2 and Table 2), but the percentage of difference is (for the specimens of *Hyalophora* calculated) highest for *H. mexicana* sp. n. versus all other *Hyalophora* (between 2.2 and 2.6%, compared with the other taxon groups, see Table 2). *H. mexicana* sequences formed a distinct basal cluster relative to all other *Hyalophora* studied, with high bootstrap support (99%). Of the other *Hyalophora*, only *H. "gloveri* b", at the second branching, has a comparable sequence distance (between 1.6 and 2.3% versus all other *Hyalophora* except *H. mexicana* sp. n.) and equal bootstrap support. For all other comparisons between populations, distance values are below 2%. Also their bootstrap values are generally low, sometimes even below 50%; only *H. "gloveri* b", *H. "gloveri* a" and *H. mexicana* sp. n. show support values above 90% (Fig. 2).

Rather low barcode differences are generally not very rare in the family Saturniidae (e.g., Nässig *et al.*, 2010; Naumann & Löffler, 2013). Often times, other characters (morphological, geographical or ecological evidence etc., but also including biological effects like reduced hybrid fertility) must additionally be taken into account to define species limits. Recent speciation does not necessarily require clear differences in all characters.

Conclusions

We cannot agree with Tuskes *et al.* (1996) who interpreted these Mexican *Hyalophora* as being conspecific with *H. columbia gloveri* (sensu lato); the differences between *H. mexicana* n. sp. and any of the other species are larger than those between any other of the presently accepted northern species of *Hyalophora*. The new species *H. mexicana* from Central Mexico does neither show all the characters of *H. columbia gloveri*, nor is it evidently the sister-taxon of that complex, in contrast to the opinion of Tuskes *et al.* (1996).

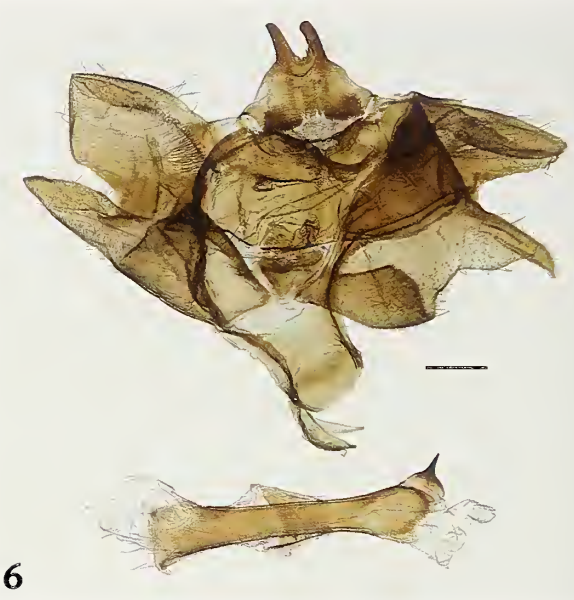


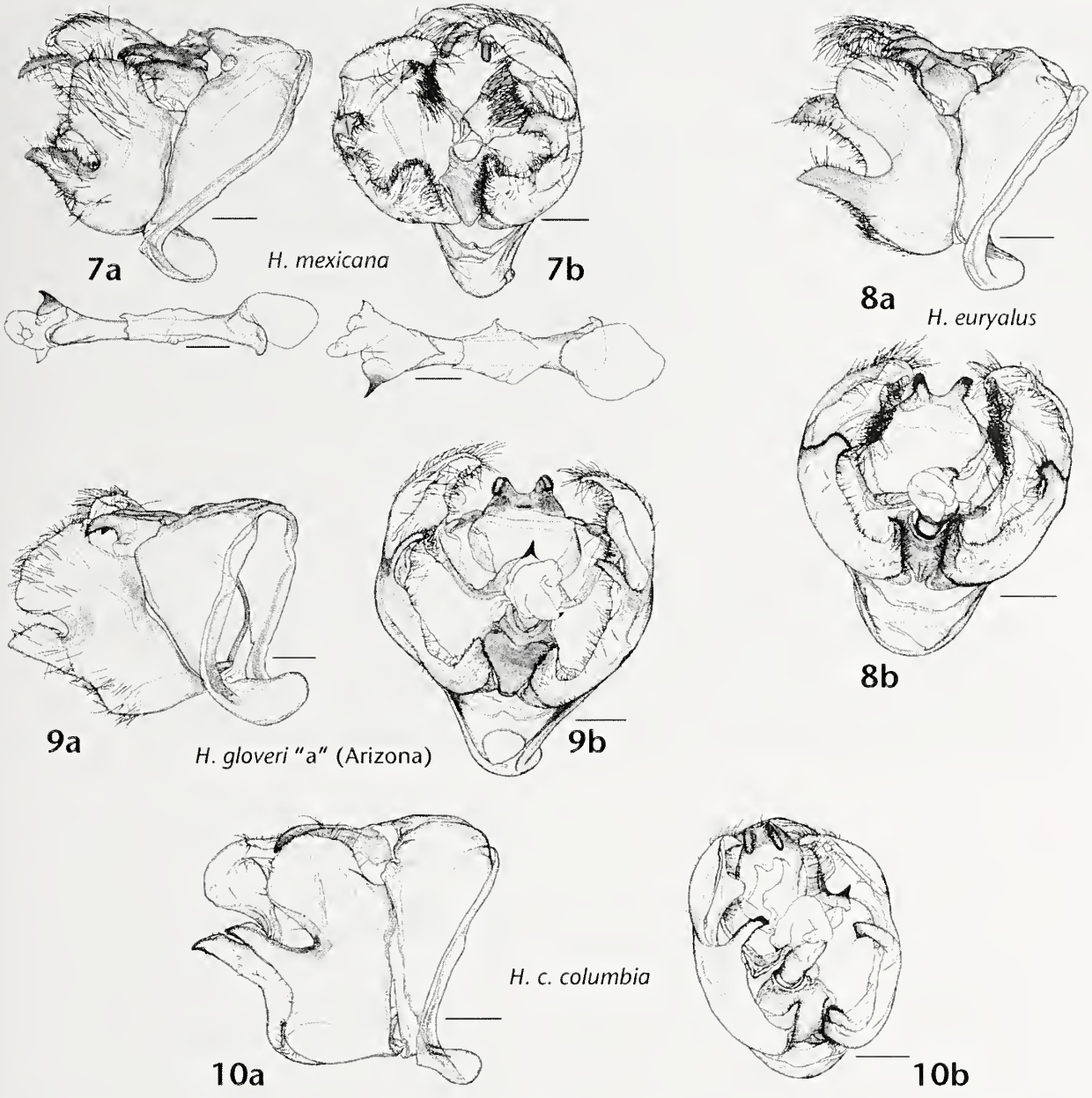
Figure 6. Photo of ♂ genitalia, *Hyalophora mexicana* sp. n., holotype, GP no. WAN 1988/07, barcode B3218-wn-B08, in INBUNAM. Scale bar = 1 mm.

H. mexicana, in spite of its similar colouration, is also clearly not conspecific with *H. euryalus* from the Pacific coastline. Possibly *H. mexicana* is a basal offshoot of the genus, at least when interpreting the barcode results in a phylogenetic way. In addition, the two, three or even four populations presently subsumed under "*H. columbia gloveri*" (sensu lato) form, based on our barcode results, quite evidently not a genetically homogeneous population and possibly may turn out to belong to two or more different species or subspecies, in spite of only rather minor differences in the barcode sequences.

Taxonomic and nomenclatural notes on some taxa

During our studies we found a few taxonomic and nomenclatural details regarding *Hyalophora* and some of its subordinate taxa to be either unclear or erroneous in other literature sources, which we correct here:

1. *Hyalophora* Duncan, 1841 (*in* Jardine [ed.], Naturalist's Library, vol. 32 = Entomology vol. 7, Exotic Moths, p. 124, 132, pl. XI): *In* Fletcher & Nye (1982) and Beccaloni *et al.* (2005/2014) the authorship for this taxon is given as "Duncan [& Westwood]"; however, these authors do not provide a source for their



Figures 7–10: Drawings of ♂ genitalia, **Hyalophora** species for comparison. **7**. *H. mexicana* sp. n., paratype, Zacatecas, GP no. WAN 1965/05, barcode B3218-wn-B09, UAG. **7a**. lateral view, phallus lateral view. **7b**. ventral view, phallus dorsal view. **8**. *H. euryalus*, GP no. WAN 1962/05, SMFL. **8a**. lateral view. 8b. ventral view. **9**. *H. c. gloveri* "a" (Arizona), GP no. WAN 1963/05, SMFL. **9a**. lateral view. 9b. ventral view. **10**. *H. columbia*, GP no. WAN 1964/05, SMFL. **10a**. lateral view. **10b**. ventral view. Drawings by Harald Lux, Berlin. Scale bars = 1 mm.

information. As Jardine's book series "The Naturalist's Library" is, first, rather rare in libraries and, second, was published under several differing cover titles (so that in different libraries slightly differing title pages and volume counts can be found for the same contents), it was not clear to us for several years whether this interpreted coauthorship was correct. However, recent digitised scans in the Biodiversity Heritage Library (general URL: www.biodiversitylibrary.org) in the internet, an original copy of the book in the private library of Colin G. Treadaway (Limbach-Wagenschwend) and photocopies from different university libraries in personal libraries (of U. Brosch, W. A. Nässig and S. Naumann), and further the papers by Stainton (1885) and de Joannis (1926) allow the following explanations and interpretations:

a. Within the book series' counting, this publication was included in volume 32, which corresponds to the Entomology volume 7. There was evidently a double volume counting, both for the entire book series and for the main parts like Entomology etc. However, the different copies of each book apparently had either a title-page with the full volume count or with the Entomology

Table 2. Estimates of divergence over sequence pairs between groups. The number of base substitutions per site from averaging over all sequence pairs between groups are shown. Standard error estimates are shown above the diagonal [in square brackets]. Analyses were conducted using the Maximum Composite Likelihood model (Tamura *et al.*, 2004). The rate variation among sites was modeled with a gamma distribution (shape parameter = 3). The differences in the composition bias among sequences were considered in evolutionary comparisons (Tamura & Kumar, 2002). The analysis involved 40 nucleotide sequences (= specimens) in groups of populations (= possibly species or subspecies) or species (3 species of Callosamia united in one group). There were a total of 658 positions (= base pairs) in the final dataset. Analyses were conducted in MEGA5 (Tamura *et al.*, 2011).

Groups/taxa	*Callosamia*	*H. cecropia*	*H. columbia*	*H. euryalus*	*H. gloveri* 'a'	*H. gloveri* 'b'	*H. mexicana*
Callosamia		[0.015]	[0.015]	[0.014]	[0.015]	[0.014]	[0.014]
H. cecropia	0.098		[0.004]	[0.005]	[0.005]	[0.005]	[0.006]
H. columbia	0.098	0.013		[0.004]	[0.003]	[0.005]	[0.006]
H. euryalus	0.092	0.016	0.017		[0.005]	[0.005]	[0.006]
H. gloveri 'a'	0.098	0.017	0.009	0.019		[0.006]	[0.007]
H. gloveri 'b'	0.096	0.016	0.019	0.017	0.023		[0.006]
H. mexicana	0.095	0.023	0.026	0.025	0.026	0.022	

volume count only; we did not see any copy with both title pages combined thus far. The volume count as povided by Fletcher & Nye (1982) and Beccaloni *et al.* (2014) as being "vol. 33", however, does in any case not appear to be correct; at least we did not find any copy of the book with an imprint of this volume number. Also, the Entomology volume count for the "Exotic Moths" as being "vol. 5", as shown in the general page of the "Naturalist's Library" in the Biodiversity Heritage Library (2014), is not supported by any additional copy of the books which we have seen; we think this is an erroneous information in BHL. This view is also supported by Westwood's letter cited by Stainton (1885).

b. Duncan regularly uses some sort of a plural "royal we" (or "*pluralis majestatis*") instead of "I" or "the author" throughout his text. This is probably just a matter of personal style, and possibly it might also have been intended to show that Duncan agreed with publications of other authors (which were sometimes referred to in short in text or footnotes), or similarly. On p. 124, where the name *Hyalophora* was introduced for the first time and its meaning in ancient Greek was explained, Duncan wrote: "... we would assign the name *Hyalophora* ..."; this page 124 should, therefore, be taken as the original description of the generic name, not p. 132. On p. 132, the text of the different species assigned by Duncan to his genus *Hyalophora* starts with *H. cecropia*. — However, this general use of the plural "we" by Duncan alone does surely not allow to interprete a coauthorship by Duncan & Westwood for *Hyalophora*.

c. Stainton (1885), based on some correspondence in letters between him and A.G. Butler as well as J.O. Westwood, clearly showed that indeed a few parts of the text in the general introduction of the series and in some of the Entomology volumes were evidently based on Westwood's results, while most others were not, with Westwood himself even correcting some of Butler's interpretations.

Butler wrote *in* Stainton (1885: 181), mainly regarding "*Scopelodes unicolor*, Westwood" (see Duncan, 1841: 222): "From an examination of the text in the volume it is evident that Duncan was supplied by Westwood with the greater part of his information, and there is every reason to believe that the characters of the new genera and species were also obtained from the same source: at page 209 you will see: '*Asthenia podaliriaria*, Westwood. In supplying us with a figure of this new species, Mr. Westwood has suggested the propriety of referring it, along with several others, to a new genus, which he names *Asthenia*.' Then follow the generic characters. The style of description corresponds entirely with Westwood's descriptive work. See 'Cabinet of Oriental Entomology' and other early works by this author. At the same time, perhaps, the question is worth ventilating; Professor Westwood probably will remember whether he wrote the descriptions for Duncan or not." However, this text by Butler is evidently only concerning two taxa in which the headline of a taxon is closed with the explicite authorship "Westwood", as for example the above cited *Asthenia* or *Scopelodes*, and a few more.

In contrast, Westwood himself clearly wrote (*in* Stainton 1885: 183) that in cases where he provided the drawings on the plates he also included a description: "... the drawings, with a popular description of each species (not, however, accompanied by a technical Latin character) were forwarded by me to Edinburgh, but, unfortunately, I never saw a proof either of the plates which contained my figures or of the text in which my descriptions were introduced by Mr. Duncan, without any indication of what was mine or what his own comments." Thus, although Westwood did provide some of the plates printed in Entomology vol. 7, he did clearly not produce pl. XI of vol. Entomology 7 illustrating *Hyalophora cecropia*. Therefore, any coauthorship by Westwood for *Hyalophora* cannot be interpreted from the painting, following Westwood's personal comment *in* Stainton (1885: 183, 185–186).

The same result (Duncan as sole author for *Hyalophora*) was also achieved by de Joannis (1926: 10), however, with an incorrect type species interpretation.

Summarised: There does not appear any reason whatsoever to interprete a combined authorship "Duncan [& Westwood]" specifically for the genus *Hyalophora*. — For any other new taxa introduced in the Entomology volume 7 of Jardine's Naturalist's Library and as well for Entomology vols. 1 and 6, only a critical reading of both Duncan's and Stainton's texts can reveal the correct authorships; a generalised "Duncan [& Westwood]" for all new taxa is surely incorrect. Westwood was, by his own statement *in* Stainton (1885: 182), explicitely not general coauthor for vols. Entomology 2–5.

Regarding the type species fixation for the genus *Hyalophora* (*i.e.*, *Phalaena cecropia* Linnaeus, 1758, by subsequent designation by Grote, 1865: 227) we follow Fletcher & Nye (1982: 79–80), who summarised the different interpretations by several authors, including Ferguson (1972: 245). Grote's earlier paper of the same year 1865, cited by Fletcher & Nye (1982), was not available to us; presently these earliest issues of the "Practical Entomologist" of 1865 are not available, neither in German libraries nor in form of digitised scan PDFs in the internet. Accepting the evidently well-argumented interpretation of Fletcher & Nye (1982), however, supports stability of nomenclature.

Figure 11. Mexico, Zacatecas. Collecting locality of *Hyalophora mexicana*.

2. *H. c. nokomis* (Brodie, 1894): In Beccaloni *et al.* (2014), the publication of Brodie is cited to be published in 1884. However, the reprint of this paper by Riotte (1970) clearly indicates an original publication date in 1894, just as well as Ferguson (1972). In earlier years we did not have access to any original copy of this very rare Canadian publication series "The Biological Review of Ontario" (and thus based our interpretation in the beginning solely on the reprint by Riotte, 1970), but in the meanwhile, since 2012, a scan PDF of this publication is available in the Biodiversity Heritage Library. So we can finally solve the question here now: the short paper by Brodie was indeed published in October 1894. — We suggest this might just have been a simple typing error in the BMNH Card Index.

3. *H. c. kasloensis* Cockerell [*in* Packard], 1914: This taxon is listed in error as "*kasloensis* Cockerell, 1908" by Beccaloni *et al.* (2014). This cited earlier paper of "Cockerell (1908)" was, in fact, written by J.W. Cockle (not by T.D. Cockerell) and does not contain a formal description of a new taxon, but only a short note and morphological description without naming of what six years later was then described by Cockerell [*in* Packard] (1914: 226, footnote) as *kasloensis*. — This was evidently a combined double error: first, by a misspelling (Cockerell instead of Cockle) while writing by hand the earlier citation onto the card "*kasloensis*" of the BMNH Card Index, but with the correct statement "as *rubra*" (i.e., without a remark on any original description), and, second, by a misinterpretation of this handwritten note while transferring the card contents into the digital Global Lepidoptera Names Index. The error may have been supported by the slightly ambivalent text by Cockerell *in* Packard (1914).

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Editor's note

The electronic edition of this article has been registered in ZooBank to comply with the requirements of the amended International Code of Zoological Nomenclature (ICZN). This registration makes this work available from its electronic edition. ZooBank is the official registry of Zoological Nomenclature

according to the ICZN and works with Life Science Identifiers (LSIDs). The LSID for this article is: urn:lsid:zoobank.org:pub:6D66A3E0-FE85-43E1-A4F3-26C473C641F4. Registration date: October 28th, 2014. This record can be viewed using any standard web browser by clicking on the LSID above.

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Evolution of extreme proboscis lengths in Neotropical Hesperiidae (Lepidoptera)

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Abstract. Exaggerated morphologies have evolved in insects as adaptations to nectar feeding by natural selection. For example, the suctorial mouthparts of butterflies enable these insects to gain access to floral nectar concealed inside deep floral tubes. Proboscis length in Lepidoptera is known to scale with body size, but whether extreme absolute proboscis lengths of nectar feeding butterflies result from a proportional or disproportional increase with body size that differs between phylogenetic lineages remains unknown. We surveyed the range of variation that occurs in scaling relationships between proboscis length and body size against a phylogenetic background among Costa Rican Hesperiidae. We obtained a new record holder for the longest proboscis in butterflies and showed that extremely long proboscides evolved at least three times independently within Neotropical Hesperiidae. We conclude that the evolution of extremely long proboscides results from allometric scaling with body size, as demonstrated in hawk moths. We hypothesize that constraints on the evolution of increasingly long butterfly proboscides may come from (1) the underlying scaling relationships, i.e., relative proboscis length, combined with the butterfly's flight style and flower-visiting behaviour and/or (2) developmental constraints during the pupal phase. Lastly, we discuss why butterflies did not evolve similar scaling relationships as hawk moths.

Key words: Skippers, hawk moths, scaling relationship, allometry, flower-visiting behaviour, metamorphosis.

Introduction

Exaggerated morphologies in animals are mainly known from traits that evolved by sexual selection and competition for access to mates, such as the antlers of elk or the horns of beetles (Emlen, 2001). Typically, these extraordinary features vary intraspecifically, so that not all individuals of a species express the trait to the same extent, and trait size often, but not always, scales with body size (Emlen & Nijhout, 2000). The slopes of the scaling relationships between the dimensions of each trait and variation in body size can vary from no slope (size-invariant trait expression), very steep slopes (traits become disproportionately larger with increasing body size) to negative slopes (traits become proportionately smaller with increasing body size; Emlen & Nijhout, 2000). Scaling relationships for morphological traits in insects have evolved and can be measured by comparing related taxa. This is because scaling relationships result from developmental processes that regulate the growth of body parts and these processes are influenced by the manner in which genotypes respond to environmental conditions during growth (for a review see Emlen & Nijhout, 2000).

Exaggerated morphologies in insects do not evolve by sexual selection alone, but also by natural selection. For example, the extremely elongate mouthparts of hawk moths, butterflies, nemestrinid flies or euglossine bees evolved as adaptations for gaining access to food resources, i.e., floral nectar concealed in deep corolla tubes (Darwin, 1862; Johnson & Steiner, 1997; Alexandersson & Johnson, 2002; Johnson *et al.*, 2002; Borrell, 2005; Pauw *et al.*, 2009; Krenn, 2010). These studies present examples of how

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adaptive departures from the usual proportional scaling relationships can represent a selective advantage in foraging (Kunte, 2007). Interspecific comparative studies on hawk moths and butterflies showed that proboscis length is correlated positively with body size (Agosta & Janzen, 2005; Corbet, 2000; Kunte, 2007), and that nectar feeding butterflies have disproportionately longer proboscides than non-nectar feeding butterflies (Kunte, 2007). Until now, there are have been no studies on the differences between the scaling relationships of butterflies with extremely long and short proboscides in relation to their phylogenetic background.

Here, we surveyed the range of variation that occurs in scaling relationships between proboscis length and body size in Neotropical Hesperiidae butterflies. We tested whether extreme absolute proboscis lengths in skippers results from a proportional increase of proboscis length and body size or from a disproportional increase, i.e., greater relative proboscis lengths. To the end, the significance of scaling relationships on the evolution of ever longer mouthparts in butterflies is discussed.

Material and methods

Study site and field work

Sampling of Hesperiidae was carried out in the garden and surroundings of the Tropical Station La Gamba (SW Costa Rica: Puntarenas Province, Piedras Blancas National Park, 8°45'N, 83°10'W; 81 m a.s.l.) in September-October 2010, September-October 2012 and January-February 2013. The Tropical Research Station is surrounded by a mosaic of habitats including primary forest, secondary forest and intensively used land (Weissenhofer *et al.*, 2008; Krenn *et al.*, 2010). Skippers were collected with a hand net and stored in 70 % ethanol. Classification of taxa follows the most recent phylogeny of Hesperiidae (Warren *et al.*, 2009).

Morphometrics

Body length and proboscis length was measured in representatives of 75 species belonging to three subfamilies of Hesperiidae (Hesperiinae: 41; Eudaminae: 17; Pyrginae: 17). The numbers of measurements for each species depended on its commonness and ease of capture, and ranged from 1 to 39. Mean body size, proboscis length and relative proboscis length (absolute proboscis length divided by body length) for each species are given in Table 1.

In the year 2010, body length and proboscis length of live specimens was measured. Skippers were cooled to approximately 20° C. Subsequently, body length of immobilized butterflies was measured with a digital caliper. The proboscis was uncoiled manually with the aid of a dissection needle, fixed with insect pins and photographed with an Olympus µ-Tough 6000 digital camera (Olympus, Tokyo, Japan). These photographs were imported to ImageJ (U.S. National Institutes of Health, Bethesda, USA) and measured with the aid of the segmented line tool.

In the years 2012 and 2013, body length and proboscis length of ethanol-preserved specimens was measured. Body length was measured by pinning the body of each specimen in a lateral position to a foam mat. After taking a micrograph of the body, the proboscis of each specimen was separated from the head at its base, uncoiled and fixed on a foam mat using insect pins. Micrographs of the body and the proboscis were taken using a Nikon SMZ 1500 stereomicroscope (Nikon, Tokyo, Japan) equipped with an Optocam-I digital camera (Nikon, Tokyo, Japan). Micrographs were imported to ImageJ and body length as well as proboscis length was measured with the aid of the segmented line tool.

Statistical analyses

We used analyses of covariance for testing if the scaling relationships between body size and proboscis length, i.e., relative proboscis length of Hesperiidae species, differs among the three subfamilies Hesperiinae, Eudaminae and Pyrginae. ANCOVA was used to test the assumption of homogeneity of slopes among these three groups. Analyses were conducted with untransformed data in the statistical package IBM SPSS Statistics 21.0 (IBM Corporation, New York, USA). Graphical illustrations were prepared using SigmaPlot 12.5 (Systat Software Incorporated, San Jose, California, USA) and CorelDRAW X6 (Corel Corporation, Munich, Germany).

Results

Body size and proboscis length were measured for a total of 370 individuals of Hesperiidae belonging to 75 species and 50 genera. Mean proboscis length per species varied eightfold between 6.4 mm and 51.8 mm, whereas mean body length per species ranged from 9.0 mm to 30.4 mm, varying only threefold (Table 1). Mean relative proboscis length also varied considerably between 0.5 (i.e., proboscis is half as long as the body) and 2.4 (i.e., proboscis is more than twice as long as the body). The longest proboscis ever discovered

Table 1. Body length, absolute proboscis length and relative proboscis length, measured in 370 individual skippers representing 75 species and 50 genera. Note: Given are mean values (± standard deviation), whenever more than one individual per species was measured.

Species	N	Body length [mm]	Proboscis length [mm]	Relative proboscis length
Eudaminae				
Astraptes fulgerator azul (Reakirt, [1867])	1	25.5	23.1	0.9
Astraptes alardus latia Evans, 1952	2	25.5 (± 2.1)	23.8 (± 0.4)	0.9 (± 0.1)
Astraptes anaphus annetta Evans, 1952	1	23.9	19.5	0.8
Astraptes brevicauda (Plötz, 1886)	1	19.8	19.7	1.0
Astraptes talus (Cramer, 1777)	1	21.7	17.8	0.8
Autochton longipennis (Plötz, 1882)	9	17.3 (± 1.3)	16.0 (± 1.3)	0.9 (± 0.05)
Autochton zarex (Hübner, 1818)	2	18.8 (± 0.3)	16.3 (± 1.5)	0.9 (± 0.1)
Bungalotis quadratum quadratum (Sepp, [1845])	1	30.4	39.4	1.3
Cogia calchas (Herrich-Schäffer, 1869)	7	14.7 (± 1.3)	11.8 (± 1.2)	0.8 (± 0.03)
Drephalys heraclides E. Bell, 1942	1	20.0	14.3	0.7
Dyscophellus porcius porcius (C. Felder & R. Felder, 1862)	1	24.9	25.5	1.0
Spathilepia clonius (Cramer, 1775)	9	19.1 (± 2.0)	15.5 (± 1.3)	0.8 (± 0.04)
Typhedanus undulatus (Hewitson, 1867)	1	16.2	12.4	0.8
Urbanus procne (Plötz, 1881)	5	18.9 (± 1.5)	15.6 (± 0.8)	0.8 (± 0.07)
Urbanus simplicius (Stoll, 1790)	16	18.7 (± 1.6)	16.3 (± 0.7)	0.9 (± 0.06)
Urbanus tanna Evans, 1952	9	20.4 (± 1.3)	16.6 (± 0.6)	0.8 (± 0.03)
Urbanus teleus (Hübner, 1821)	13	18.3 (± 1.5)	15.9 (± 0.9)	0.9 (± 0.04)
Pyrginae				
Pyrrhopygini				
Mysoria ambigua (Mabille & Boullet, 1908)	4	23.2 (± 1.0)	15.3 (± 0.6)	0.7 (± 0.03)
Pyrrhopyge phidias evansi E. Bell, 1947	1	27.5	15.9	0.6
Celaenorrhini				
Celaenorrhinus darius Evans, 1952	1	21.1	29.8	1.4
Celaenorrhinus monartus (Plötz, 1884)	1	15.4	20.4	1.3
Erynnini				
Chiomara mithrax (Möschler, 1879)	1	15.4	10.6	0.7
Ebrietas osyris (Staudinger, 1876)	1	19.5	11.8	0.6
Pyrgini				
Pyrgus orcus (Stoll, 1780)	3	13.7 (± 0.3)	8.1 (± 0.1)	0.6 (± 0.01)
Xenophanes tryxus (Stoll, 1780)	3	11.7 (± 0.2)	8.5 (± 1.7)	0.7 (± 0.1)
Achlyodini				
Achlyodes busirus heros Ehrmann, 1909	1	19.6	13.3	0.7
Milanion marciana Godman & Salvin 1895	1	13.3	9.4	0.7
Oileus panna Evans, 1953	1	11.7	10.6	0.9
Carcharodini				
Nisoniades ephora (Herrich-Schäffer, 1870)	1	15.2	10.1	0.7
Nisoniades godma Evans, 1953	3	14.7 (± 0.3)	10.5 (± 0.3)	0.7 (± 0.03)
Nisoniades rubescens (Möschler, 1877)	3	14.8 (± 0.4)	10.0 (± 0.7)	0.7 (± 0.1)
Noctuana stator (Godman, 1899)	1	16.8	8.9	0.5
Staphylus ascalaphus (Staudinger, 1876)	1	10.9	8.4	0.8

Table 1. (Cont.)

Species	N	Body length [mm]	Proboscis length [mm]	Relative proboscis length
Staphylus carribea (Williams & E. Bell, 1940)	4	11.2 (± 0.9)	8.0 (± 0.2)	0.7 (± 0.05)
Hesperiinae				
Clade 113				
Lycas godart boisduvalii (Ehrmann, 1909)	1	25.7	45.7	1.8
Perichares adela (Hewitson, 1867)	8	23.2 (± 1.5)	44.5 (± 4.9)	1.9 (± 0.1)
Perichares lotus (A. Butler, 1870)	1	22.8	48.3	2.1
Pyrrhopygopsis socrates orasus (H. Druce, 1876)	1	26.1	34.4	1.3
Calpodini				
Aroma henricus henricus (Staudinger, 1876)	4	20.9 (± 1.6)	29.9 (± 1.8)	1.4 (± 0.04)
Calpodes ethlius (Stoll, 1782)	6	24.6 (± 2.5)	39.8 (± 3.9)	1.6 (± 0.04)
Carystoides escalantei H. Freeman, 1969	5	23.2 (± 1.1)	33.2 (± 1.5)	1.4 (± 0.09)
Carystoides hondura Evans, 1955	2	22.7 (± 1.4)	28.9 (± 0.3)	1.3 (± 0.1)
Damas clavus (Herrich-Schäffer, 1869)	20	23.4 (± 1.9)	49.5 (± 2.1)	2.1 (± 0.1)
Damas immaculata Nicolay, 1973	2	22.1 (± 2.0)	52.0 (± 1.0)	2.4 (± 0.2)
Panoquina ocola ocola (W. H. Edwards, 1863)	14	16.3 (± 0.9)	13.7 (± 0.5)	0.8 (± 0.05)
Saliana esperi esperi Evans, 1955	8	18.6 (± 1.0)	36.5 (± 2.5)	2.0 (± 0.2)
Saliana longirostris (Sepp, [1840])	1	26.4	42.7	1.6
Saliana salius (Cramer, 1775)	3	23.3 (± 0.6)	47.2 (± 5.7)	2.0 (± 0.2)
Saliana severus (Mabille, 1895)	1	29.7	51.8	1.8
Saliana triangularis (Kaye, 1914)	9	20.9 (± 1.5)	41.1 (± 2.1)	2.0 ± (0.1)
Talides hispa Evans, 1955	2	25.0 (± 1.5)	45.0 (± 0.7)	1.8 (± 0.1)
Talides sergestus (Cramer, 1775)	1	22.1	36.6	1.7
Thracides phidon (Cramer, 1779)	1	27.0	42.0	1.6
Tromba xanthura (Godman, 1901)	1	20.9	48.2	2.3
Anthoptini				
Anthoptus epictetus (Fabricius, 1793)	6	11.9 (± 0.8)	12.9 (± 0.4)	1.1 (± 0.08)
Anthoptus insignis (Plötz, 1882)	1	12.0	12.2	1.0
Corticea lysias lysias (Plötz, 1883)	7	12.4 (± 0.9)	12.6 (± 1.1)	1.0 (± 0.04)
Moncini				
Apaustus gracilis gracilis (C. Felder & R. Felder, 1867)	6	9.0 (± 0.7)	6.4 (± 0.7)	0.7 (± 0.07)
Arita arita (Schaus, 1902)	1	18.8	27.4	1.5
Callimormus radiola radiola (Mabille, 1878)	6	9.9 (± 0.4)	9.0 (± 0.5)	0.9 (± 0.06)
Cymaenes alumna (A. Butler, 1877)	7	12.9 (± 0.9)	15.9 (± 0.9)	1.2 (± 0.09)
Cymaenes tripunctus theogenis (Capronnier, 1874)	1	16.9	20.3	1.2
Flaccilla aecas (Stoll, 1781)	1	15.1	20.0	1.3
Lerema ancillaris (A. Butler, 1877)	1	16.0	20.5	1.3
Mnasilus allubita (A. Butler, 1877)	3	11.2 (± 0.02)	12.8 (± 0.6)	1.1 (± 0.1)
Mnasitheus chrysophrys (Mabille, 1891)	1	10.1	9.3	0.9
Morys geisa (Möschler, 1879)	39	14.6 (± 1.2)	20.2 (± 1.4)	1.4 (± 0.09)
Morys micythus (Godman, 1900)	8	15.6 (± 0.9)	19.1 (± 1.2)	1.2 (± 0.07)
Papias phaeomelas (Hübner, [1831])	21	14.5 (± 1.3)	19.3 (± 4.0)	1.3 (± 0.2)
Papias phainis Godman, 1900	2	13.3 (± 0.6)	16.3 (± 0.2)	1.2 (± 0.1)
Papias subcostulata (Herrich-Schäffer, 1870)	29	17.3 (± 1.2)	24.8 (± 2.6)	1.4 (± 0.1)

Table 1. (Cont.)

Species	N	Body length [mm]	Proboscis length [mm]	Relative proboscis length
Vehilius stictomenes illudens (Mabille, 1891)	6	12.4 (± 1.0)	13.1 (± 0.9)	1.1 (± 0.05)
Vettius marcus (Fabricius, 1787)	1	14.6	21.4	1.5
Hesperiini				
Pompeius pompeius (Latreille, [1824])	14	15.5 (± 1.0)	14.5 (± 0.8)	0.9 (± 0.06)
Quinta cannae (Herrich-Schäffer, 1869)	7	18.8 (± 1.2)	21.7 (± 1.1)	1.2 (± 0.06)

in butterflies thus far was in a specimen of *Damas immaculata* Nicolay, 1973 (Hesperiinae: Calpodini) and measured 52.7 mm. Several individuals had proboscides measuring more than 50 mm, such as specimens of *Damas clavus* (Herrich-Schäffer, 1869) (Hesperiinae: Calpodini), *Perichares adela* (Hewitson, 1867) (Hesperiinae: Clade 113), *Saliana salius* (Cramer, 1775) (Hesperiinae: Calpodini) and *Saliana severus* (Mabille, 1895) (Hesperiinae: Calpodini). The shortest proboscis measuring only 5.3 mm was found in a representative of the species *Apaustus gracilis gracilis* (C. Felder & R. Felder, 1867) (Hesperiinae: Moncini).

Proboscis lengths of 75 species were categorized according to the quartiles of the data range as (1) short: ≤ 12.6 mm (first quartile), (2) medium: > 12.7 to ≤ 17.8 mm (second quartile), (3) long: > 17.9 to ≤ 29.9 mm (third quartile) and (4) extremely long: > 30.0 mm (fourth quartile; see Figure 1). 70 % of the species representing the subfamily of Hesperiinae were characterized by long (12 out of 41 species) and extremely long (17 out of 41 species) proboscides. By contrast, most Pyrginae had short proboscides (12 out of 17 species). Within Eudaminae, medium sized proboscides were most abundant (9 out of 17). Extremely long proboscides occurred within Hesperiinae, but also in a single species of Eudaminae.

Within all three subfamilies, proboscis length increased with increasing body length (Hesperiinae: $F_{(1, 39)} = 184.3$, $p < 0.0001$; Eudaminae: $F_{(1, 15)} = 83.0$, $p < 0.0001$; Pyrginae: $F_{(1, 15)} = 7.3$, $p < 0.05$). The regression slopes of the three subfamilies differed significantly (Figure 2). For every 1 mm body length gain, proboscis length increased by 2.4 mm within Hesperiinae, by 1.5 mm within Eudaminae and by 0.7 mm within Pyrginae.

Hesperiinae had the steepest slope, indicating that these butterflies had disproportionately long proboscides, i.e., higher relative proboscis lengths. Within Hesperiinae, two groups (Calpodini and clade 113) had the highest relative proboscis lengths (mean = 1.8) and departed from the isometric scaling relationships of other Hesperiinae such as Moncini (mean = 1.2), Anthoptini (mean = 1.0) and Hesperiini (mean = 1.1).

Discussion

Longest proboscis among butterflies found within Hesperiidae

Among insects, the world record holder concerning absolute proboscis length is *Amphimoea walkeri* (Boisduval [1875]) (Sphingidae). The proboscis of this Neotropical hawk moth measures up to 280 mm (Amsel, 1938). Among butterflies, the standing record regarding proboscis length has been held by the riodinid butterfly *Eurybia patrona* Staudinger, 1876. Its proboscis measures up to 49.9 mm (Kunte, 2007). In addition, exceptionally long proboscides were noted in at least four genera of Hesperiidae (Kunte, 2007). Here, we provide further evidence that Hesperiidae comprise many species with exceptionally long proboscides. Further, we now have a new record holder for absolute proboscis length in butterflies: *D. immaculata* with a proboscis length of up to 52.7 mm.

Evolution of extremely long proboscides

Mapped onto a cladogram (Warren *et al.*, 2009), we conclude that extremely long proboscides among Neotropical Hesperiidae presumably evolved at least three times independently (Figure 3), once within the subfamily Eudaminae and twice within groups of Hesperiinae: viz. Hesperiinae-Calpodini, and Hesperiinae-clade 113 (Table 1). Nearly all members of the tribe Calpodini analysed in this study were characterized by long or even extremely long proboscides, except *Panoquina ocola ocola* (W. H. Edwards, 1863), which had a medium-sized proboscis measuring only 13.7 mm on average. However, it is possible that other extremely long-proboscid species could also be found among Palaeotropical Hesperiidae. By contrast, extremely long proboscides in butterflies outside of the Hesperiidae are known to occur only within a single genus of Riodinidae, *Eurybia* (Kunte, 2007; Bauder *et al.*, 2011; Bauder *et al.*, 2013).

Our data showed that each of the three investigated skipper subfamilies Hesperiinae, Eudaminae and Pyrginae featured a characteristic scaling relationship between body size and proboscis length, i.e., relative proboscis length. Hesperiinae had the steepest slope, indicating that these butterflies had disproportionately long proboscides. Therefore, extreme absolute proboscis lengths in skipper butterflies are the result of allometry (slope of regression line: 2.4 for Hesperiinae) and do not scale isometrically with body size (slope of regression line would be 1.0).

What prevents butterflies from evolving even longer mouthparts?

The evolution of extreme absolute proboscis lengths in skipper butterflies is closely linked to extreme relative proboscis lengths, since body size and absolute proboscis length scaled allometrically. In hawk moths, the extreme proboscis length of *Amphimoea walkeri*, 280 mm, corresponds to the fourfold of body length (Amsel, 1938), whereas our present data and those of former studies (Kunte, 2007; Bauder *et al.*, 2011; Bauder *et al.*, 2013) showed that relative proboscis length in butterflies never exceeds 2.5. These results indicate that proboscis length in hawk moths can exceed that of butterflies not only because hawk moths are larger, but also because of a steeper scaling relationship between body size and proboscis length. Two not mutually exclusive explanations for what keeps butterflies from evolving equally long mouthparts in relation to body size as hawk moths could be found in differences regarding the flower-visiting behavior and/or metamorphosis.

A crucial difference between butterflies and hawk moths regards their flower-visiting behavior: hawk moths typically hover over or in front of flowers during nectar uptake (Farina *et al.*, 1994), whereas nearly all butterflies need to sit on the flower to feed (Krenn, 2008), except for Troidini (Papilionidae). In butterflies, uncoiling a very long proboscis is limited by how far a butterfly can bend back its head and stretch its legs to allow for straightening of the proboscis spiral while sitting on the flower. None of these problems apply to hawk moths, which can modulate the space needed for uncoiling by hovering at an acceptable distance in front of or over the flower. Although absolute proboscis length determines access to nectar in flowers with deep tubes, relative proboscis length plays a crucial role during the uncoiling process and might constrain butterflies from evolving even longer mouthparts.

Further, developmental constraints could limit the evolution of proboscis length in butterflies since proboscis formation takes place in a developmental sheath on the ventral side of the pupa (Lowe *et al.*,

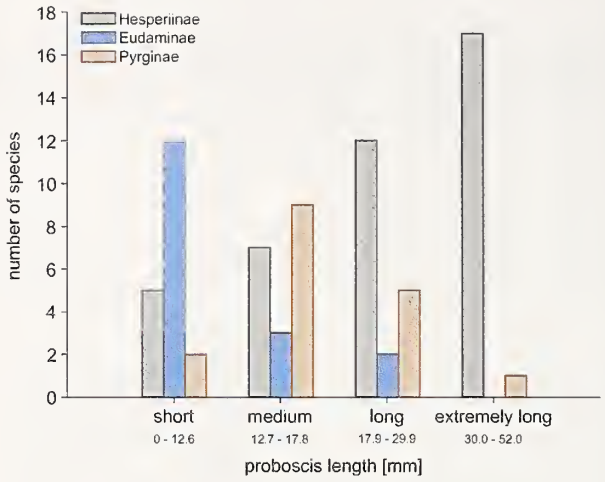

Figure 1. Categorization of proboscis lengths measured in 75 species representing three subfamilies of Hesperiidae (Hesperiinae, Eudaminae, Pyrginae) according to quartiles of data range: short: ≤12.6 mm; medium: 12.7 to 17.8 mm; long: 17.9 to 29.9 mm; and extremely long: 30.0 to 52.0 mm.

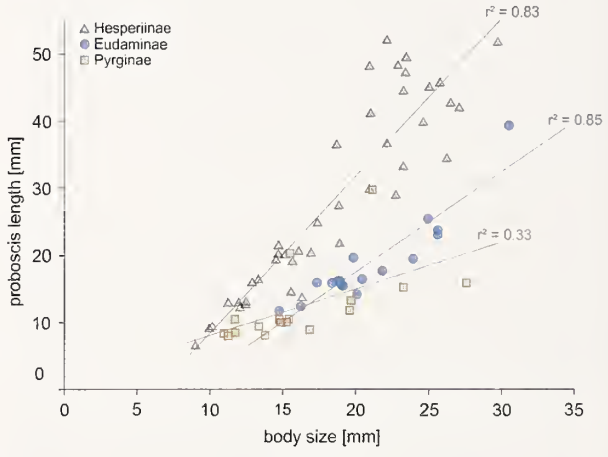

Figure 2. The allometric relationship between body size and proboscis length in Costa Rican Hesperiidae butterflies. Hesperiinae (N = 41 species) had significantly longer proboscides for a given body size compared to Eudaminae (N = 17 species) or Pyrginae (N = 17 species). Regression lines were fitted as: Hesperiinae: $y = 2.4x - 15.1$; Eudaminae: $y = 1.5x - 12.3$; and Pyrginae: $y = 1 + 0.7x$. Scaling relationships differed significantly among the three subfamilies (ANCOVA, homogeneity of regression slopes, Hesperiinae-Eudaminae: $p < 0.05$; Eudaminae-Pyrginae: $p < 0.05$; Hesperiinae-Pyrginae: $p < 0.0001$).

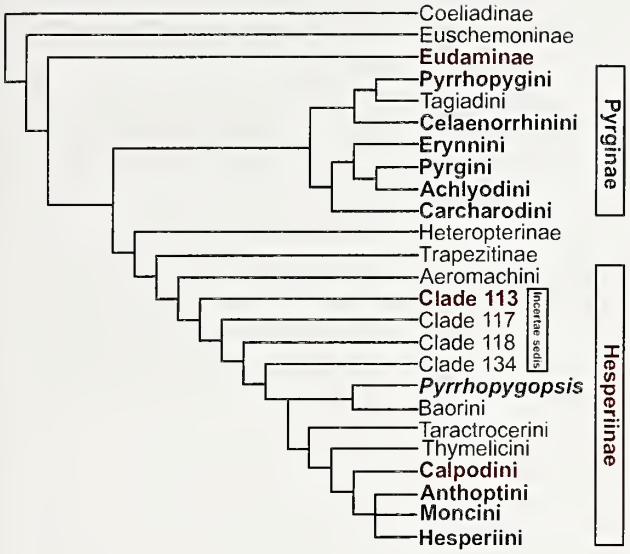

Figure 3. Simplified cladogram of the family Hesperiidae (Warren *et al.*, 2009). Extremely long proboscides evolved at least three times independently within Neotropical Hesperiidae in representatives of the subfamilies Eudaminae and two tribes of Hesperiinae. Note: Taxa printed in bold are represented in this study, taxa printed in red include species with extremely long proboscides that exceed 30 mm in length.

2013), where the galeae are straight and arranged parallel to each other. Since the developmental sheath contains the full length of the unfolded proboscis, this organ grows accordingly to accommodate the extreme length of the adult proboscis and may extend a full body length beyond the last abdominal segment (Figure 40A, p. 137: DeVries, 1997). Further elongation of this fragile and thin pupal organ might constrain proboscis length evolution in butterflies. By contrast, the pupae of long-proboscid hawk moths during metamorphosis develop a heavily sclerotized, hook-shaped external outgrowth that contains a loop of the developing proboscis that allows for the formation of a proboscis of much greater length (Patočka, 1993).

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Arawacus euptychia (Lepidoptera: Lycaenidae: Eumaeini) is no longer an obscure species

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Abstract. The taxonomy, morphology, distribution, and habitat of the poorly-known *Arawacus euptychia* are discussed. Sampling for one year in the Atlantic Forest of Northeast Brazil was conducted, and we obtained basic information about this species. The previously unknown female is described. Its genitalia bear papillae anales that are subterminally constricted, which supports the placement of this species in *Arawacus*. Adults are illustrated, and their wing patterns are compared with those of sympatric euptychiine butterflies from the nymphalid subfamily Satyrinae.

Key words: Butterfly, Brazilian Northeast, Euptychiina, butterfly wing pattern, false head hypothesis.

Introduction

The South American lycaenid butterflies *Arawacus euptychia* (Draudt, 1920) and *Evenus satyroides* (Hewitson, 1865) (Theclinae, Eumaeini) possess wing patterns that resemble those of *Euptychia* Hübner and other Satyrinae (Nymphalidae), as indicated by their specific names. Whereas *E. satyroides* is a widespread and relatively common species (Draudt, 1919-1920), *A. euptychia* is one of the more obscure butterfly species in South America. Although Draudt (1919-1920: 811) described *Thecla euptychia* "according to a ♂ from the Museu Paulista from South Brazil," the type specimen is not extant (Mielke & Casagrande, 1988; M. Duarte pers. comm.), and the species has not been recorded in lists of the south Brazilian butterfly fauna (e.g., Ebert, 1969; K. Brown, 1992; Duarte *et al.*, 2010). Indeed, no specimens were known until a putative male was found in 1991 by the junior author in the remote mountains of Bahia, Brazil (an image of this male was published in D'Abrera, 1995: 1137). Robbins (2000) transferred *Thecla euptychia* to *Arawacus* Kaye based on similarity between the genitalia of this male and of the male of *Arawacus tadita*. However, generic placement was provisional because the sole morphological synapomorphy for *Arawacus* was the shape of the female papillae anales (Robbins, 2000). *Arawacus euptychia* was later recorded in a montane semi-deciduous forest fragment in Pernambuco, northeastern Brazil (Paluch *et al.*, 2011).

This paper has the following purposes related to the name *Arawacus euptychia*: 1) to explain the basis for the identification of the species; 2) to illustrate the morphology of the female; 3) to present evidence from the female morphology that confirms the generic placement of *A. euptychia* and that shows that this species is likely most closely related to *A. dumenilii* and *A. tadita*; 4) to document the distribution, habitat, and flight behavior of *A. euptychia*; and 5) to assess the biological significance of the resemblance in wing pattern between *A. euptychia* and sympatric Satyrinae butterflies.

Materials and methods

For the dissection of the female genitalia, the abdomen was boiled in a solution of 10% potassium hydroxide for five minutes. The genitalia were examined with a Leica EZ4D stereomicroscope and photographed with a built in digital camera. The terminology of the genitalia follows Klots (1970), as modified for the Eumaeini (Robbins, 1991). Wing vein names follow Comstock (1918), and the abbreviations FW and HW are used for forewing and hindwing, respectively.

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The following acronyms are used for museum collections where specimens of *A. euptychia* are deposited or that are mentioned in the text.

CE-UFPE — Coleção Entomológica, Universidade Federal de Pernambuco, Recife, Brazil
DZUP — Museu de Entomologia Pe. Jesus Santiago Mourem, Universidade do Paraná, Curitiba, Brazil
MF-CE — Coleção Entomológica do Museu de Fauna/ Cemafauna, Universidade Federal do Vale do São Francisco, Petrolina, Brazil
MZUSP — Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil
SMF — Forschungsinstitut und Naturmuseum Senckenberg, Frankfurt am Main, Germany
USNM — National Museum of Natural History, Smithsonian Institution, Washington, DC, United States

Although the distribution of *A. euptychia* was based on specimens deposited in the collections just listed, most specimens of and information about *A. euptychia* resulted from a butterfly survey along the borders and understory trails (4:1 sampling hours) of six Atlantic Forest remnants located at the Usina São José S/A (USJ) (coordinates: 7°41'4.09" to 7°54'41.6"S; 34°05'17.6 to 35°05'07.2"W), a sugarcane industry facility situated in the municipality of Igarassu, northeastern Pernambuco, Brazil (Fig. 1). Butterflies were sampled from four to six days per month, from April/2007 to March/2008 for a total of approximately 300 net-sampling hours. This northeastern portion of Atlantic Forest is characterized as dense lowland rainforest (Veloso *et al.*, 1991) and has a more seasonal rainfall regime than the southeastern portion (Schessl *et al.*, 2008). The fragment sizes were from 12 to 387 ha with elevations ranging from 20 to 155 m a.s.l. The mean annual temperature from 1998 to 2007 was 24.9° C. Sampling time was from 08h00 to 12h00, and from 13h00 to 16h00, between April 2007 to March 2008. In the first six months, only four of the six fragments were sampled. Data on temperature and precipitation were available from weather stations operated by the USJ. The specimens from this survey are deposited in the entomological collection at CE–UFPE.

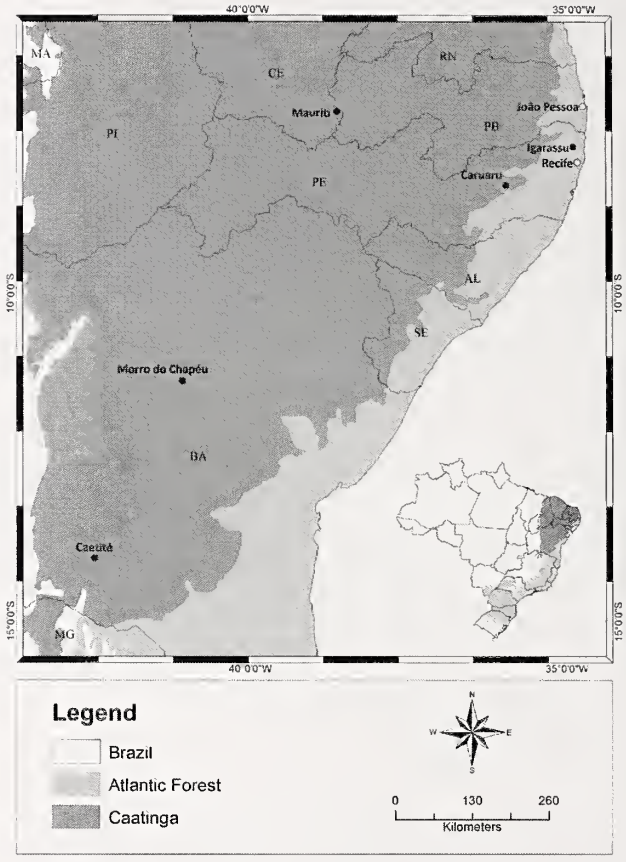


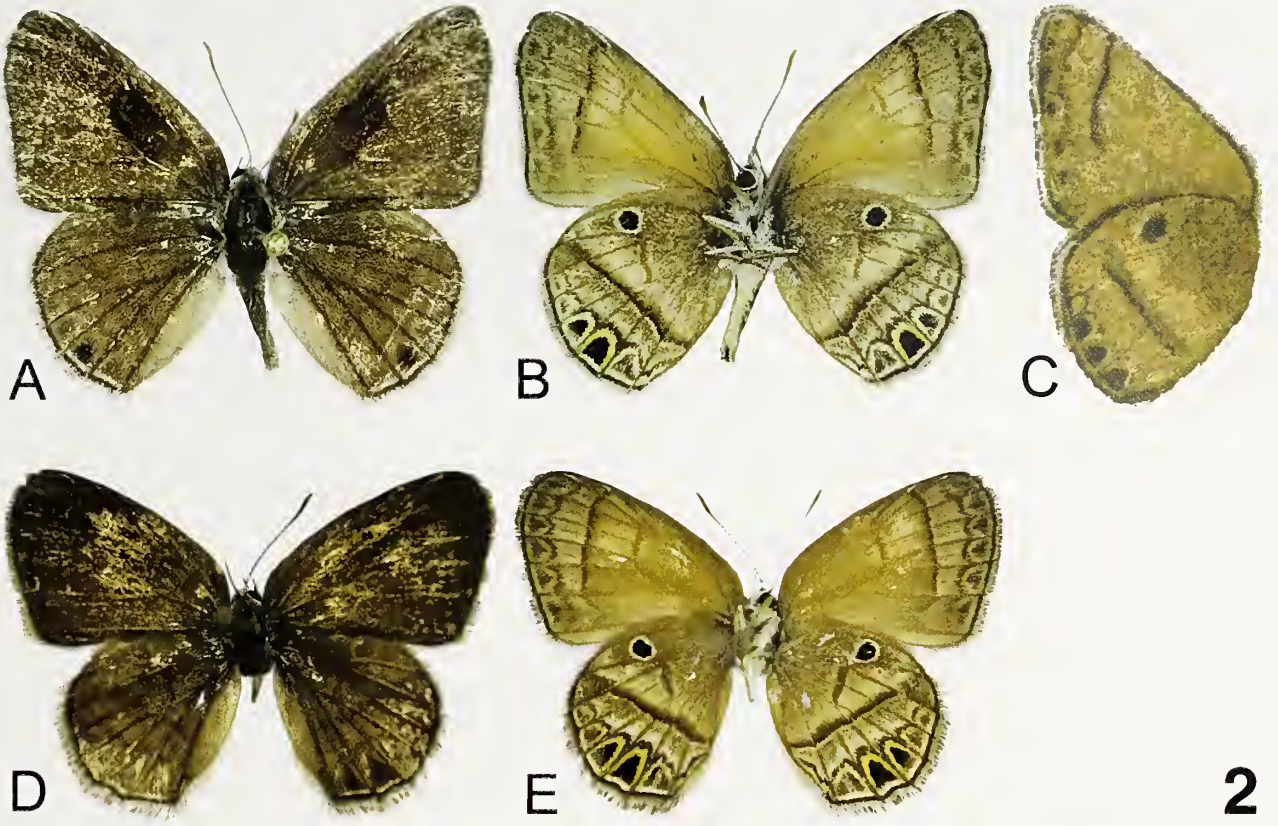
Figure 1. Distribution map for *Arawacus euptychia*. Black dots – occurrence localities; white dots – localities from which *A. euptychia* is unrecorded despite extensive survey work.

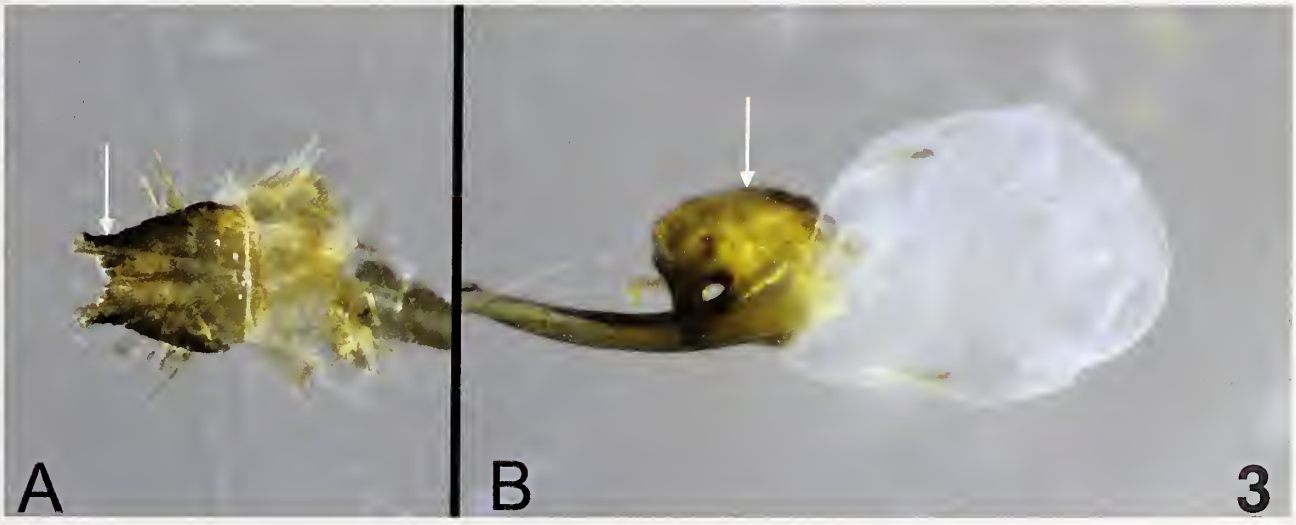
Results and discussion

Identification of the name *Thecla euptychia* Draudt

Draudt (1919-1920) described *Thecla euptychia*, *T. pura*, and *T. clarissa* from specimens in the "São Paulo museum." The types of the latter two names are in MUZSP, but the type of the first is not (Mielke & Casagrande, 1988). The current curator of Lepidoptera, Marcelo Duarte, confirms that there is no type of *T. euptychia* in MZUSP. Many Draudt types in the Lycaenidae are deposited in Frankfurt (SMF), but the type of *T. euptychia* is not there either (Lamas & Robbins, in prep.). Without a type, the only information about this name is that contained in the original description.

The detailed original description of *Thecla euptychia* (Draudt, 1919-1920: 811) follows. "**Th. euptychia** *spec. nov.* (145 1) has the shape of *tadita*, but it is all brown with a black scent-spot, on the hindwing analwards a white border-line and between the median veins a small black spot bordered proximally with a light colour. Beneath the disc of the forewing is rusty yellow, distally brown-grey, the hindwing brown-grey, with the same marking as in the preceding, the costal-marginal eye-spot very large, jet-black, white-ringed, also the spot between the median veins and one above it jet-black, upwards with a golden yellow ring."





Figures 2-3. 2. Adults of *Arawacus euptychia*. Male: (A) dorsal, (B) ventral, (C) original illustration in Draudt (1920); female: (D) dorsal, (E) ventral. **3**. Female genitalia of *Arawacus euptychia*. (A) dorsal aspect of papillae anales, posterior to left, showing subterminal constriction (arrow). (B) lateral aspect of ductus copulatrix, posterior to left, showing sclerotized pouch from which the ductus seminalis arises (arrow).

This written description fits the illustrated male (Figs. 2A, 2B) in all respects. In particular, the combination of rusty yellow at the base of the ventral forewing and the golden yellow ring around the submarginal spot on the ventral hind wing is definitive because it describes no other species of *Arawacus* or Eumaeini, so far as we are aware. We base our identification of the name primarily upon the written text because the original illustration was poor (Fig. 2C).

Female of *Arawacus euptychia*

Label: Igarassu - PE, Usina São José, Pezinho. Brasil, 22.05.2007. C.E.B. Nobre, leg. (Figs. 2D-2E).

Diagnosis. The female differs externally from the other sex only by the rounded apex of forewing and by the more triangular-shaped marginal eye-spots on the hindwing. **Wings**: FW length: 11.4 mm. Color pattern identical to that of males: dorsal ground color brown, border dark brown, fringe brown, medially light. HW with marginal white ray on Cu_1 and Cu_2. Ventral ground color cream, with rusty yellow FW basal half. Two brown bars on both FW and HW: one postmedian from veins R_2 to Cu_1 and the other on distal margin of discal cell. FW with faint submarginal brown spots bordered by a light brown and a dark brown line, on cells R_2 to Cu_1. HW with distinct postmedian band formed by white and brown scales and medial black spot bordered by white scales on cell Rs. Black somewhat triangular spots bordered proximally by yellow and then, brown scales and distally only by brown scales on cells M_3 and Cu_1, the last one being larger. **Head**: Antennae (5.6 mm): brown, on the stalk ringed with white scales at base of each segment, but not on the dorsal surface. Nudum confined to the club. Eyes naked, golden brown bordered by white scales. Palpi white with scattered brown scales and last segment brown. **Body**. Thorax dorsally brown, ventrally white. Legs white, except on tibia and tarsi, which are tan.

Genitalia (Fig. 3): Tips of papillae anales black, heavily sclerotized, subterminally constricted at base (Fig. 3A), setae concentrating medially. Corpus bursae with a pair of hollow-pointed signa, longer than wide, projecting anteriorly; ductus seminalis arising from a pouch, dorsal to the ductus bursae, and fused to it anteriorly; ductus bursae bent at middle (Fig 3B).

Systematic placement of *Arawacus euptychia*

Robbins (1991, 2000) distinguished *Arawacus* morphologically by subterminally constricted papillae anales and ecologically by caterpillars that eat the leaves of *Solanum* (Solanaceae). Although the larval food plant of *A. euptychia* remains unknown, the shape of the papillae anales is subterminally constricted (Fig. 3A, arrow). Further, the shape and sclerotization of the pouch of the corpus bursae (Fig. 3B) from which the ductus seminalis arises is indistinguishable from that in *A. dumenilii* and *A. tadita*. In *A. binangula*, the pouch is of a similar shape, but it is not as sclerotized as in *A. euptychia*. Finally, wing pattern elements

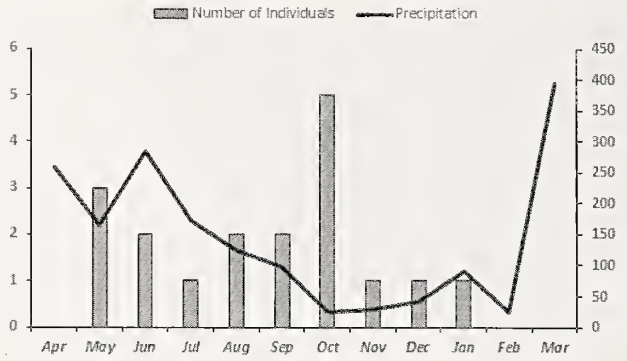


Figure 4. Number of individuals of *Arawacus euptychia* and monthly precipitation from April 2007 to March 2008 in the municipality of Igarassu, northeastern Pernambuco, Brazil.

of *A. dumenilii* and *A. tadita* are similar of those of *A. euptychia*. These results are consistent with the placement of *A. euptychia* in *Arawacus*, and it is likely that *A. dumenilii*, *A. euptychia*, and *A. tadita* form a clade within *Arawacus*.

Seasonality, distribution, habitat, and flight behavior of *A. euptychia*

In one year of sampling in the municipality of Igarassu, 18 individuals were recorded. The only female found was collected in a 30 ha remnant, at an approximate elevation of 100 meters. Species abundance was low throughout the year, and no obvious correlation was found between the number of individuals and monthly precipitation (Fig. 4).

The known distribution of *A. euptychia* is restricted to northeastern Brazil, but this species occurs in a variety of habitats. It has been recorded in montane Caatinga (Bahia: Caetité at 850 m elevation, Morro do Chapeu at 1200 m and Ceará: Mauriti at 808 m), coastal Atlantic Forest near sea level (Pernambuco: Igarassu), and montane Atlantic Forest (Pernambuco: Caruaru, 8°21'45.36" S, 36°02'11.31" W at approximately 840 m, Paluch *et al.*, 2011). The last locality is called "brejo de altitude" (altitude wetland), a site of humid forest surrounded by semi-arid Caatinga vegetation (Andrade-Lima, 1982). Despite the varied habitats in which *A. euptychia* has been found, its occurrence is hard to predict. For example, it was not recorded in two large urban forest remnants of Paraíba (Mata do Buraquinho, 7°08'42" S, 34°51'34" W; Kesselring & Ebert, 1982) and Pernambuco (Parque Estadual Dois Irmãos, 8°00'31" S, 34°56'53 W; D.H. Melo,

Table 1. Species of Euptychiina sympatric with *Arawacus euptychia* in the state of Pernambuco, Brazil. [1]Atlantic Forest (C.E.B. Nobre, unpublished); [2]Montane wetland (Paluch *et al.*, 2011); [3]Caatinga (Nobre *et al.*, 2008).

Species	Locality		
	Igarassu[1]	Caruaru[2]	Catimbau National Park[3]
Chloreuptychia arnaca (Fabricius, 1776)		x	
Cissia myncea (Cramer, 1780)	x	x	
Cissia palladia (A. Butler, 1867)	x		
Cissia terrestris (A. Butler, 1867)	x		
Erichthodes antonina (C. & R. Felder, 1867)	x		
Euptychoides castrensis (Schaus, 1902)	x		
Hermeuptychia atalanta (Butler, 1867)		x	
Hermeuptychia gr.hermes (Fabricius, 1775)	x		x
Magneuptychia libye (Linnaeus, 1767)	x	x	
Pareuptychia ocirrhoe interjecta (D'Almeida, 1952)	x	x	
Paryphthimoides poltys (Prittwitz, 1865)		x	
Pharneuptychia innocentia (C. & R. Felder, 1867)			x
Pharneuptychia phares (Godart, [1824])			x
Yphthimoides affinis (A. Butler, 1867)	x	x	
Yphthimoides manasses (C. & R. Felder, 1867)		x	
Yphthimoides ochracea (A. Butler, 1867)		x	
Yphthimoides renata (Stoll, 1780)	x	x	
Zischkaia sp.	x		

pers. comm.), although both areas are similar in their vegetation to the forest remnants of northern Pernambuco (Fig. 1).

Wing pattern of *A. euptychia* and sympatric satyrine butterflies

Unlike many *Arawacus* and other Eumaeini species, the wings of *A. euptychia* do not possess the components of a "false head" wing pattern (Robbins, 1980, 1981). Rather, the ventral wing pattern of this species is more like that of sympatric euptychiine butterflies (Nymphalidae: Satyrinae: Euptychiina) in the state of Pernambuco (Table 1), especially the genus *Pharneuptychia*. This genus occurs in open habitats and forest edges, and is one of the few Euptychiina that are common in dry forests, such as the Caatinga. In particular, the species *P. phares* and *P. innocentia* (Nobre *et al.*, 2008; Kerpel *et al.*, 2013) are about the same size as *A. euptychia* and have rusty yellow scales at the base of the ventral forewing.

The flight behavior of *A. euptychia* also appears to be similar to that of euptychiine butterflies. DeVries (1987) described the flight of euptychiines as "a characteristic bouncy flight," but in contrast, we would describe the typical flight of eumaeine lycaenids as rapid and directed. The male of *A. euptychia* from Morro do Chapeu was flying through short grass at the edge of a dirt road. It was flying with satyrine butterflies, and was flying like a typical satyrine. In Igarassu, the adults were found at forest borders, in sunny sites.

Conclusions

Arawacus euptychia was an obscure species because it occurs in the northeast of Brazil, where the butterfly fauna is more poorly documented than in the south, and because its occurrence within its range appears to be unpredictable. Female morphology provides evidence that it is correctly placed in *Arawacus* and that it is closely related to *A. dumenilii* and *A. tadita*. The characters given in the original description accurately distinguish *A. euptychia* from these two relatives. The wing pattern, habitat, and flight behavior of *A. euptychia* seem to closely match that of sympatric satyrines, especially those in the genus *Pharneuptychia*. Perhaps there is an advantage to resembling common species in a habitat, such as *Pharneuptychia*, but otherwise, the reasons why *A. euptychia* would have a euptychiine-like wing pattern are yet to be determined.

Acknowledgements

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